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Biogastranema New Genus (Nematoda: Trichostrongylidae) from the California Jackrabbit, *Lepus californicus californicus* Gray (Mammalia: Leporidae)*

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In conjunction with a research project on stomach-worms of ruminants a series of collections of nematodes from the stomach of *Lepus californicus californicus* Gray, 1837, were made during 1947 and 1948. Contrary to expectations the common stomach-worm of rabbits, *Obelescoides cuniculi* (Graybill, 1923) was not found. However, two new species were represented for which a new genus is proposed.

Family: TRICHOSTRONGYLIDAE Leiper, 1912
Biogastranema new genus

DIAGNOSIS: Head nude. Body marked with strong longitudinal striae and fine transverse striae. Cervical papillae at region of posterior half of esophagus. Excretory pore anterior to cervical papillae. Cervical alae absent. Genital apparatus of female typical of trichostrongylids with two closely approximate stout muscular ovejectors and short vagina. Vulva not protected by cuticular valve. Bursa bilateral. Accessory membrane present. Bursal formula: ventral rays arising from a common stalk, directed posteriorly, but curving slightly anteriorly, terminating at bursal margin; lateral rays arising from a common stalk, antero-and medio-laterals closely approximate while dorso-lateral separates at base running parallel with externo-dorsal; externo-dorsal strong, originating at base of dorsal, curved medially; dorsal ray arising from a single stalk, dividing near midpoint into two lateral and medial branches, medial branches bifurcate, the medial branches of which may be bidigitate. Spicules simple and filiform. Gubernaculum absent. Prebursal papillae present.

TYPE SPECIES: *Biogastranema leporis* new species

DESCRIPTION: Holotype-Male. Length 12.6 mm. Widths: head 57 u; at junction of esophagus and intestine 180 u; maximum width 282 u; at

*These nematodes were first collected during a joint project of the California Fish and Game Laboratory under Dr. C. M. Herman and Dr. M. A. Stewart of the Department of Entomology and Parasitology of the University of California. The authors wish to thank Miss Doris Kleberger and I. Barry Tarshis for their aid in the collection of material and Drs. D. P. Furman, M. A. Steyart and E. G. Linsley for their suggestions in the organization of this paper. Dr. E. W. Price and Mr. J. T. Lucker of the United States Department of Agriculture, Zoological Division, very kindly checked the manuscript.

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prebursal papillae 238 u. Esophagus 813 u long, maximum width 106 u. From anterior end: nerve ring 352 u; excretory pore 458 u; cervical papillae 483 u. Spicules 1052 u long, maximum width 22 u. Bursa conforming with generic description.

HOST: *Lepus californicus californicus*
LOCATION: Stomach
LOCALITY: Napa Airport, Napa Co., California
DATE: 22 February 1949
COLLECTOR: Doris Kleberger and authors
TYPE SPECIMEN: Deposited in the United States National Museum Helminthological Collection #48626

PARATYPES: Allotype-Female. Length 12 mm. Widths: head 48 u; at junction of esophagus and intestine 150 u; at vulva 220 u. Esophagus 800 u long, maximum width 114 u. From anterior end: nerve ring 330 u; excretory pore 374 u; cervical papillae 405 u. Vulva 3.1 mm. from posterior end. Anus 176 u from posterior end. Deposited in the U. S. N. M. Helminthological collection, Allotype #48627 and Paratypes #48628 (three males and four females).

Range of 12 males: 8.1-10.8 mm. long. Widths: at head 51-60 u; at junction of esophagus and intestine 132-202 u; at prebursal papillae 176-244 u. Esophagus 686-838 u long, maximum width 88-123 u. From anterior end: nerve ring 264-308 u; excretory pore 350-429 u; cervical papillae 383-489 u. Spicules 889-1118 u long, maximum width 20-30 u.

Range of 14 females: 9.7-16.8 mm. long. Widths: at head 53-70 u; at junction of esophagus and intestine 136-233 u; at vulva 220-286 u. Esophagus 677-927 u long, maximum width 75-114 u. From anterior end: nerve ring 308-382 u; excretory pore (20-50 u anterior to cervical papillae) 400-508 u; cervical papillae 431-528 u. Vulva 2.8-4.9 mm. from posterior end. Anus 141-234 u from posterior end. Some females show a prevulvar swelling, ranging from slight to quite strong.

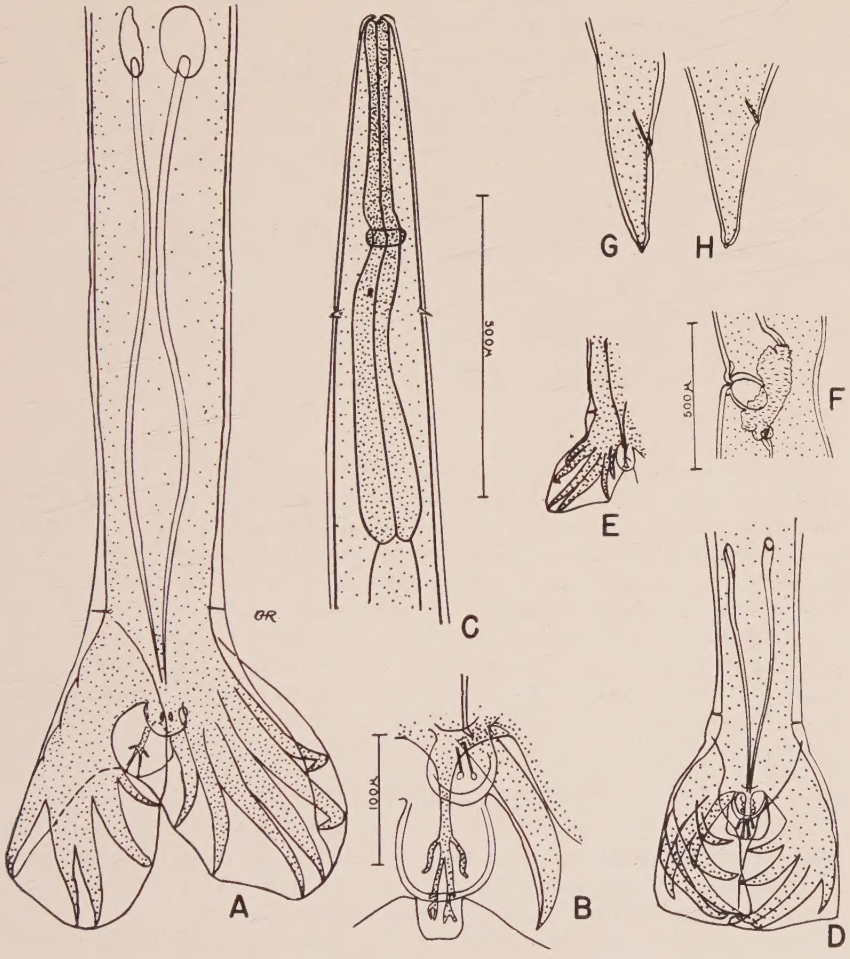
One of the males in the paratype series is from the type collection. The data for the allotype and the remainder of the paratype series are: Host, location and locality the same as for the type; Date: April 1948; Collector: I. Barry Tarshis.

Biogastranema affinis new species

DESCRIPTION: Holotype-Male. Length 7.9 mm. Widths: at head 44 u; at junction of intestine and esophagus 128 u; maximum width 185 u; at prebusal papillae 154 u. Esophagus 660 u long, maximum width 74 u. From anterior end: nerve ring 308 u; excretory pore 365 u; cervical papillae 533 u. Spicules 418 u long, maximum width 17 u. Bursa conforming with generic description.

HOST: *Lepus californicus californicus*
LOCATION: Stomach
LOCALITY: Gridley Game Refuge, Butte Co., California
DATE: 13 May 1948
COLLECTOR: I. Barry Tarshis
TYPE SPECIMEN: Deposited in U. S. N. M. Helminthological Collection #48629.

PARATYPES: Allotype-Female. Length 12.3 mm. Widths: at head 56 u; at junction of esophagus and intestine 163 u; at vulva 238 u. Esophagus 838 u long, maximum width 119 u. From anterior end: nerve ring 352 u; excretory pore 532 u; cervical papillae 584 u. Vulva 3.3 mm. from posterior end. Anus 154 u from posterior end. Tail blunter than *B. leporis*. Deposited in the U. S. N. M. Helminthological collection Allotype #48630, and Paratypes #48631 (one male) and #48632 (2 males and 2 females).



A. *Biogastranema leporis* nov. sp. bursa of holotype.
B. *Biogastranema leporis* nov. sp. dorsal ray.
C. *Biogastranema leporis* nov. sp. anterior end of holotype.
D. *Biogastranema affinis* nov. sp. bursa of holotype.
E. *Biogastranema affinis* nov. sp. view of bursa extended.
F. *Biogastranema affinis* nov. sp. ovejectors.
G. *Biogastranema affinis* nov. sp. posterior end of allotype.
H. *Biogastranema leporis* nov. sp. posterior end of allotype.

Range of 33 males: Length 6.5-10.1 mm. Widths: at head 44-84 u (in four specimens there was a distinct swelling at the head region, but this did not appear to be typical); at junction of esophagus and intestine 74-198 u; maximum width 180-304 u; at prebursal papillae 163-229 u. Esophagus 546-890 u long, maximum width 75-110 u. From anterior end: nerve ring 308-458 u; excretory pore 365-486 u; cervical papillae 394-572 u. Spicule length 338-635 u (in some cases spicules not equal but differ in length up to 20 u; in one case they varied 102 u), maximum width 17-27 u.

Range of 18 females: Length 10.9-14.9 mm. Widths: at head 44-79 u; at junction of esophagus and intestine 141-330 u; at vulva 229-396 u. Esophagus 711-953 u long, maximum width 92-286 u. From anterior end: nerve ring 352-444 u; excretory pore 489-624 u (40-80 u anterior to cervical papillae); cervical papillae 546-667 u. Vulva 2.5-4.4 mm. from posterior end. Anus 154-308 u from posterior end.

Three males and one female in the paratype series are from the type collection. Collection data for the allotype, 17 females and 11 males are: same host, location and locality as type; Date: 24 April 1948, Collector: I. Barry Tarshis. Collection data for 17 males are: same host and location as type; Locality: Napa Airport, Napa Co., California; Collector: F. A. Ehrenford.

In addition to the material deposited in the U. S. N. M. Helminthological collection, paratypes have also been deposited in the University of California collection and in the authors' collections.

Discussion

Biogastranema closely resembles the genera *Citellinoides* Dikmans, 1949, *Citellinema* Hall, 1916, (as redefined by Dikmans, 1948) and *Graphidium* Ralliet and Henry, 1909 (as redefined by Travassos, 1937), but may be readily separated from these three genera by the presence of the accessory membrane of the male bursa and the absence of a cephalic cuticular inflation. In addition, *Biogastranema* can be separated from *Graphidium* by the absence of a gubernaculum, from *Citellinema* and *Citellinoides* by prominent cervical papillae, ovejectors close to vulva, and absence of a "spike" on female's tail.

All measurements were taken from relatively undistorted mounted specimens of the type series. The measurement ranges demonstrated in the paratype series indicate that there is a fairly wide range of variation found within these two species. In the present study there was found no direct correlation between worm size and size of internal structures within the species.

Both species were found in the stomach proper and occasionally a few were recovered from the anterior duodenum close to the gastric pyloric valve. No gross pathology was observed which was attributable to those nematodes except for petechial hemorrhages near the recovered worms.

Both glycerine jelly and C-M medium (Clark and Morishita 1950) were used in mounting specimens. It was noted that specimens mounted in C-M medium were cleared better than those subjected to lacto-phenol and then mounted in glycerine jelly. It was also found that specimens mounted in C-M medium should not be dried at a temperature higher than 35° C as they tend to overclear and become too flattened. This medium appears to be ideal for the temporary mounting of small nematodes as they can be mounted directly from alcohol and need not be ringed. Since the medium is rather slow drying, specimens may be rotated for location of structures for about 48 hours after mounting. It has been our experience that delicate specimens

may have to be first infiltrated with a 1:1 mixture of 70 per cent alcohol and the medium to prevent collapsing of the specimen. However, it was also observed that nematode specimens in C-M medium tended to overclear after a period of six months or more making it difficult to see delicate structures. Thus the C-M medium should be restricted in use to temporary mounts of nemas for clarification of morphology not readily seen in glycerine jelly mounts.

SUMMARY

Rather than encountering the species *Obelescoides cuniculi,* two new species of Trichostrongylidae were found in the stomach of the California jackrabbit, *Lepus californicus californicus.* A new genus, *Biogastranema,* is erected for the two new species described in this paper, *B. leporis* and *B. affinis.* Information is given for the use of C-M medium in the mounting of small nematodes.

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A Case of Conjoined Twins in *Loxothylacus* (Crustacea, Rhizocephala)

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The occurrence of conjoined twins in the Rhizocephala is apparently very rare. The only instance on record is the double monster of *Sacculina carcini* studied by Pérez and Basse (1928). The present case is therefore noteworthy particularly since the details of the doubling differ somewhat from those of the sacculinid described by the French authors and because for the first time serial sections were employed in the study.

The specimen in question is a parasite of the crab *Panopeus herbstii* Milne Edwards collected in 1941 near Englewood, Florida and encountered in a lot of parasitized mud crabs received from the Zoological Research Supply of Englewood. Attached by a single stalk to the abdomen of the crab are twin sacs of *Loxothylacus panopei* (Gissler) in close lateral union but each with its own mantle opening (fig. 1). This species of Rhizocephala is a common parasite of crabs of the family Xanthidae in the Gulf of Mexico and the Caribbean region (Boschma, 1928).

In the Sacculinidae, the family to which both Sacculina and Loxothylacus belong, the surface of the parasite that is in contact with the thorax of the host is its left side; the surface that lies against the abdomen of the crab is its right side. The dorsal region of the parasite, determined by the position of the mesentery, lies at the right side of the crab, while the ventral or anti-

mesenterial region falls on the left side of the host (See Boschma, 1948). Anterior and posterior correspond with the region of the mantle opening and the region of the stalk, respectively. The orientation of the twin sacs described here is in agreement with this rule and the terms given above will be employed in their usual sense. To distinguish, however, between the two members of the pair we shall use the term "inner" for the parasite that is in contact with the thorax of the host and "outer" for the twin that faces the abdomen of the crab.

OBSERVATIONS

Externally it can be noted that the outer sac is somewhat smaller than the inner one and that the two are in contact along their entire lateral surface except at the extreme dorsal and ventral angles. The left side of the outer sac is joined to the right side of the inner sac, with a shallow furrow marking the line of union. The single stalk of attachment is connected with the inner animal. Two well-developed mantle openings, rather widely separate from each other, protrude from the surface opposite the point of attachment. In general appearance, except where joined together, the sacs have the characteristic shape of the species. The combined sacs measure 4 mm. in length, 8 mm. in dorso-ventral diameter and 4 mm. in breadth. They are therefore not dwarfed specimens but conform in length and thickness with the average size of adult sacs of *Loxothylacus panopei.*

From a complete series of traverse sections cut at 10μ and stained with hematoxylin and Biebrich scarlet details of the internal anatomy could be readily worked out.

The sections show that a thin longitudinal muscular septum completely separates the two sacs from each other in their dorsal portions (fig. 2). This septum is an inward reflection of the muscular portion of the mantle wall and is covered on both sides with a continuation of the inner cuticle of the mantle cavity. The position of the partition corresponds with the furrow that is visible externally.

As the septum approaches the mid-region it becomes incomplete and the posterior attachment is lost (fig. 3). In the region between the two mantle openings it is a short tongue of tissue hanging down from the anterior roof of the cavity (fig. 4). The partition gradually diminishes as it proceeds ventrally and in the ventral third there is no longer any remnant of it except the indentation in the exterior body wall (fig. 5).

Accordingly, where the septum is complete, the visceral masses of the twin animals are completely distinct, with each enclosed in its own mantle cavity. In the region of the stalk and mantle openings the two visceral masses are united at their posterior ends and the mantle cavities become confluent. In the ventral region where the septum is absent the two visceral masses are fused posteriorily along half their length and the mantle cavity is an uninterrupted chamber.

In the genus Loxothylacus the visceral mass lies obliquely in the mantle cavity. This is due to the fact that the mesentery is ordinarily attached to the mantle a short distance to the right of the stalk. This relationship is clearly shown in figure 4. The twin visceral masses are not dircetly united to the single stalk, as they are in the Sacculina described by Pérez and Basse, but meet the body wall directly to the right of the stalk. It appears therefore that only the inner sac of the twin Loxothylacus possesses a stalk, although this stalk feeds both visceral masses. Whether the single stalk represents a

Fig. 1. Double monster of *Loxothylacus panopei* parasitic on the crab *Panopeus herbstii*. The specimen was photographed in situ and is shown here attached to the underside of the abdomen of the host. The dorsal portion of the parasite is directed to the left of the figure.

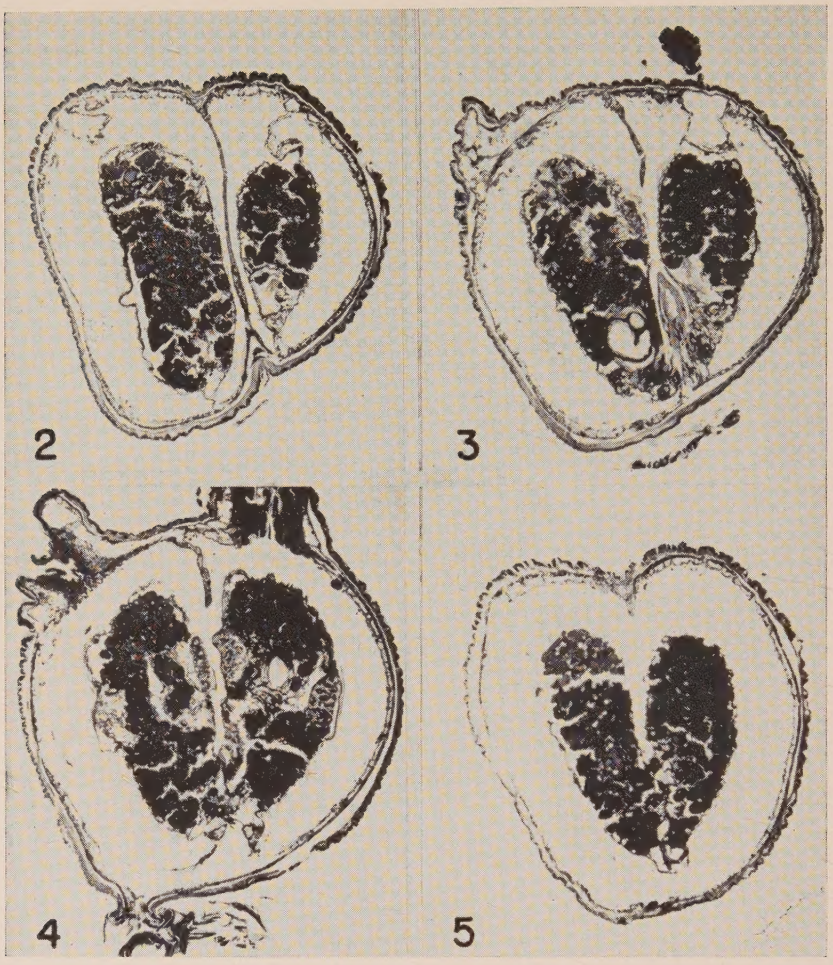
fusion of two stalks or whether it originated solely from the inner animal cannot be determined from the material at hand.

Despite the fact that the two visceral masses are joined together, each retains its identity with respect to the reproduction organs. Each animal has a separate set of testes, vasa deferentia and colleteric glands and there is no crossing over of these organs from one to the other member of the pair. In the larger twin the testes appear normal and contain sperm. No sperm is present in the testes of the other sac and these organs are relatively small compared iwth the testes of the larger sac. In the inner animal the left testis is noticeably larger than the right but this is not unusual, since in *Loxothylacus panopei* one of the testes, as a rule, is somewhat larger than the other. In the smaller animal the right testis appears to be slightly better developed than the left one.

The colleteric glands show no structural abnormalities but the glands on the sides facing each other are farther forward in position than those on the opposite side, which means that in the inner twin it is the left gland and in the outer twin the right gland that is located more anteriorily (fig. 4).

DISCUSSION

It is well-known that in cases of conjoined twins in vertebrates one member of the pair is a normally developed individual while the other frequently shows some marked defects.The weaker member of the pair is often smaller and, in extreme conditions, may appear as a mere small appendage of its twin. It is customary to refer to such double monsters as "autosite-parasite" twins. The conjoined twins of *Loxothylacus panopei* show to some extent the same type of relationship since one member of the pair is smaller and has the male reproductive organs poorly developed.



Figs. 2-5. Representative transverse sections of the double monster of *Loxothylacus panopei*. (Photomicrographs taken by Henry F. Mengoli.)

2. Section through the approximate middle of the dorsal half, showing complete partition separating the two sacs. The inner (and larger) sac is to the left.

3. Section through the mid-region where the separating partition is incomplete. The two protuberances in the upper part of the figure are the edges of the muscular thickenings that surround the mantle openings. In the visceral mass at the left, normal testes and vasa deferentia may be seen. The visceral mass at the right shows vasa deferentia and rudimentary testes.

4. Section through the mid-region which passes through the stalk and both mantle openings. Note the short partition extending inward from the roof of the cavity and the attachment of the twin visceral masses to the mantle to the right of the stalk. The right and left colleteric glands may be seen in each of the visceral masses.

5. Section through ventral half. Here the septum is represented only by a notch in the mantle wall and the two visceral masses are fused posteriorly.

In vertebrate autosite-parasite pairs, as shown by the extensive work of Lynn (1946) on fishes, it is common to find also that symmetry reversal extending even in some cases to complete mirror-imaging occurs. The double monster of Loxothylacus appears to exhibit some evidence of mirror-imaging since it is the left testis of one and the right testis of the other that is larger while the left colleteric gland of one but the right colleteric gland of the other is displaced forward. The structural simplicity of Rhizocephala, however, as compared with fishes, and the chance that the peculiarities may be due to normal variability prevent accepting the evidence as conclusive.

The conjoined twins of Loxothylacus resemble the double monster of Sacculina described by Pérez and Basse (1928) in general respects. In both cases the sacs are more intimately united near their ventral regions than at the mesenterial or dorsal sides. The visceral masses in each case have paired testes and paired colleteric glands. In the Sacculina specimen, however, the visceral masses remain completely separate while in the Loxothylacus twins the two are partially united except in the dorsal half.

Pérez and Basse studied their specimen by dissection only and therefore comparisons based on microscopic anatomy are not possible. These authors did, however, observe some egg masses in the mantle cavity of both members of the pair of twins. Although no eggs or embryos were present in the mantle cavity of the twins we studied, the ovaries were packed with large eggs and it is probable that twinning likewise in this case did not seriously interfere with propagation of young.

With respect to the origin of conjoined twins, this could occur through fusion of two originally separate embryos or by a process of fission of a simple embryonic mass. Pérez and Basse explain the origin of the double monster studied by them according to the latter assumption. They think it probable that some unknown circumstance, possibly of a traumatic nature, produced a cleavage of the undifferentiated cellular mass composing the so-called "nucleus" of the tumor during an early stage in the endoparasitic development of the Sacculina. This interpretation seems satisfactory also as an explanation for the conjoined twins of Loxothylacus.

SUMMARY

A double monster (the second case reported in the Rhizocephala) of the sacculinid parasite *Loxothylacus panopei* (Gissler) occuring on the crab *Panopeus herbstii* Milne Edwards is described and illustrated by means of serial sections.

Mature twin sacs, laterally united, are attached to the host by a single stalk. An internal partition separates the two sacs in their dorsal portions, but elsewhere the mantle cavities are confluent and the visceral masses are partly joined together. Each twin has a complete and separate set of reproductive organs.

These conjoined twins exhibit an autosite-parasite relationship and some evidence of symmetry reversal.

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Albinism in *Australorbis glabratus*

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In the course of studies on the inheritance of susceptibility in the snail, *Australorbis glabratus,* to infection with *Schistosoma mansoni* (Newton, 1953), data were also obtained on the inheritance of albinism in this snail. Two strains of the latter were used in these studies; one strain was obtained originally from Puerto Rico and the other from Bahia, Brazil. These strains had been maintained in this Laboratory by Dr. E. G. Berry for several months prior to their use in the studies mentioned above.

Individuals of the Puerto Rican strain had a dark, blackish-brown coloration of the mantle and head-foot region. There was an apparent variation in the amount of pigmentation; some snails were obviously darker than others. Members of the Brazilian strain were similarly pigmented, although the color was usually a lighter brown than that of the Puerto Rican strain. There was also some shade variation in this strain, and certain individuals could not be distinguished from members of the Puerto Rican strain.

At very infrequent intervals, a red snail appeared in the Brazilian colony. Preliminary observations with snails isolated upon hatching had confirmed Brumpt's (1941) earlier conclusions that *A. glabratus* can self- as well as cross-fertilize. Viable, fertile snails were produced from eggs laid by such isolated individuals. Thus, at the request of the author, one of these snails was isolated by Dr. Berry for the purpose of establishing a colony. While some of the first offspring from this snail were pigmented, all subsequent offspring were red. A colony of this variety has now been maintained for 4 years, and no pigmented snails have appeared.

The red color of the mutant snails appears to be due to the red color of the blood. While yellow or red pigment may be present, there is no evidence of the darker type, and it is felt that the red snail is an albino. No albinos were observed among the Puerto Rican snails during these studies.

EXPERIMENTAL

Several pairings were made between recently-hatched pigmented Puerto Rican and red Brazilian snails. After several weeks, when egg production began, the members of a pair were separated. Offspring derived from each member were then observed for color.

From the pigmented member of a pairing, only pigmented offspring were obtained. This was true of the offspring from the first eggs laid after separation from the red snail, and of all subsequent offspring. On the rather likely assumption that the pigmented condition was dominant, and in view of Dr. Berry's observations to this effect with a color variety of a strain of *A. glabratus* from Venezuela, one could not determine whether a particular offspring was the result of self- or cross-fertilization until a subsequent generation. Thus, this side of the cross was not followed further.

With regard to the progeny of the red member of a pairing, in some instances these consisted entirely of red snails. Other red parents first produced pigmented snails after separation from pigmented mates, and

these were eventually followed by red snails only. From still other pairings, both kinds of offspring were produced at first and then only red snails appeared. While several explanations for such sequences were possible in view of the fact that either self- or cross-fertilization could have occurred, it was apparent that pigmented offspring from a red snail were the result of crossing. These snails will be referred to as "F_1".

Many of the F_1 from five crosses were isolated singly a few days after hatching. Forty of these produced offspring. The latter, which will be referred to as "F_2", were the result of self-fertilization. It will be apparent from the data given below that parthenogenesis did not occur.

The color distribution of the F_2 from each of the five crosses is summarized in Table 1. It can be seen that the ratio of pigmented to red snails agrees quite closely with a 3:1 hypothesis. Statistical analysis revealed that in only one cross, No. III, did the evidence against this hypothesis border on significance, ($.10>P>.05$). However, this divergence was occasioned by a single F_2 population of this cross in which the ratio of pigmented to red snails was significantly higher than 3:1. The reason for this, if other than the result of chance, was not apparent inasmuch as the distribution in the other three F_2 populations of this cross and that of all 36 F_2 populations of the other crosses could be explained by the 3:1 hypothesis.

TABLE 1.—The color distribution of F_2 derived from self-fertilized pigmented F_1 of crosses between red and pigmented *Australorbis glabratus*

Cross No.	Number of selfing F_1's*	Total Number of F_2	Observed distribution		Expected in 3:1 hypothesis	
			Pigmented	Red	Pigmented	Red
I	8	1,472	1,092	380	1,104	368
II	2	412	310	102	309	103
III	4	627	490	137	469	158
XXV	7	1,323	980	343	992	331
XXIX	19	4,447	3,324	1,123	3.335	1,112
Totals	40	8,281	6,196	2,085	6,209	2,072

*All pigmented

These findings indicated that albinism in *A. glabratus* is inherited as a simple Mendelian recessive. Additional studies with F_3 progeny bore this out. From self-fertilized red F_2's, only red offspring were obtained. The pigmented F_2's could be divided into two groups on the basis of the color of their offspring. Of 34 selfed pigmented F'_2 from the various crosses, 11 produced pigmented snails only. Each of the other 23 pigmented F_2's produced both pigmented and red offspring in a ratio of 3:1, respectively. Thus, the ratio of approximately 1:2 between those pigmented F_2's producing pigmented snails only and those producing both pigmented and red offspring is also in keeping with the single factor hypothesis. The 11 were homozygous pigmented, and the 23 were heterozygous.

The few previous studies on the inheritance of albinism and pigmentation in snails have indicated the same type of inheritance. The observations of Stelfox (1915), though rather limited, indicated that brown coloration in *Helix aspera* behaved as a simple dominant over white. Boycott and Diver (1927), in studies with isolated *Lymnaea peregra,* found that the albino mutant which appeared after several generations of selfing of pigmented snails was inherited as a simple Mendelian recessive. They stated that no melanin could be found in the body of the albino, which had a yellow

color; the wild-type color was yellowish-gray. Ikeda (1937), in a study of self-fertilization in the slug, *Philomycus bilineatus,* found that the inheritance of three pigmented longitudinal bands on the mantle followed the pattern of simple Mendelian dominance.

Studies with other animals, both vertebrate and invertebrate, have demonstrated that a single factor usually separates the albino from the pigmented animal. However, the amount, color, and pattern of pigmentation have often been shown to be under the influence of several additional genetic factors. While information on complementary factors was not obtained in the present study, it is felt that such factors exist in *A. glabratus.* As mentioned earlier, there was some color variation in the parental pigmented snails. Variation in pattern and degree of pigmentation was quite marked among the F_2 and F_3 obtained in this study. Snails varied from those with a heavily pigmented, almost black, appearance, through several intergrades, to those with a lightly pigmented and sometimes spotted appearance. However, it was difficult to separate the various types into satisfactorily objective phenotypes, and thereby gain accurate information on the inheritance of additional pigmentation factors.

The albino character should provide an excellent opportunity for certain studies on relationships between *A. glabratus* and *S. mansoni.* As indicated earlier (Newton, 1953), it can serve as a marker for genetic studies. Also, the transparency of most of the body and shell of this variety permits observation of the parasite in certain areas of the live snail. During the studies mentioned above, the author observed miracidia of the parasite during their penetration of, and sometimes migration proximad from, the tentacles. Sporocysts attached to the mantle were studied and often exhibited undulating and contracting motions. Daughter sporocysts and cercariae were observed moving about relatively freely in certain areas. While these observations were essentially incidental, they were sufficiently impressive to suggest that certain phases of the development and behavior of the parasite could be followed in the live snail, and that such a study might yield some interesting and hitherto unacquired information.

Summary

Crosses between albino snails of a Brazilian strain and pigmented snails of a Puerto Rican strain of *Australorbis glabratus,* and the subsequent self-fertilization of F_1 and F_2 progeny, gave results which indicated that albinism is inherited as a simple Mendelian recessive in this snail. The existence of other genetic factors controlling the amount and pattern of pigmentation appears likely. The use of the albino variety of the snail in certain studies on relationships between *A. glabratus* and *Schistosoma mansoni* is discussed.

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Experimental Attempts to Infect Calves With *Neoascaris vitulorum*

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The mode of infection of calves with *Neoascaris vitulorum* has given rise to considerable speculation. The only experimental data recorded is Brumpt's (1922) account of his failure to infect three calves by direct feeding of eggs. The occurrence of mature *N. vitulorum* in calves as young as 10 days to 6 weeks old has been recorded by Griffiths (1922), Boulenger (1922), Molintas (1938) and others. In view of the fact that a closely related ascarid, *Ascaris lumbricoides,* requires about 8 weeks to attain sexual maturity in swine, it has been suggested by the foregoing authors and by Schwartz (1925) and Vaidyanathan (1949) that calves must acquire their infections with *N. vitulorum* prenatally.

To the authors' knowledge, neither direct nor prenatal infection with *N. vitulorum* has heretofore been accomplished experimentally. It is our chief purpose to record an instance of experimental infection of a calf with this nematode.

The information which follows was given in abstract form in the Journal of Parasitology, *39*(4, Sec. 2):33, under the title, "Prenatal Infection of a Calf with the Nematode, *Neoascaris vitulorum.*"

MATERIALS AND METHODS

Eggs were obtained from a 3-month-old calf at Angleton, Texas, and from 7 calves in the vicinity of Jeanerette, Louisiana. The calves from the latter area were 3 months old when they were passing large numbers of eggs. Eggs were recovered from the feces by centrifugal sedimentation followed by flotation in a saturated salt solution. They were cultured in tap water in petri dishes kept at 28° C. Since Schwartz (1922) reported that *N. vitulorum* eggs cultured for 14 days were infective to guinea pigs, cultures at least that old were used for infection experiments. In addition, feeding eggs to mice in each instance of attempted infection of calves and cattle showed that eggs cultured as long as 280 days were still infective.

Grade Jersey calves purchased from local dairies within 24 hours of birth were used in attempts to infect the natural host. The calves were kept in individual pens in barns or outside in portable pens, and were given a ration of grain and alfalfa hay, in addition to while milk. Embryonated eggs were fed to the calves in water, mixed with moist rumen contents, or dry feed. When fed in water, the eggs were administered through a blowpipe attached to a 5 cc. rubber bulb.

In view of the work of Tiner (1949) and Sprent (1953) who showed that rodents could serve as intermediate hosts in the life cycles of *A. columnaris* and *A. devosi,* and despite the fact that the feeding habits of calves would appear to preclude the rodent as a possible intermediate host, *N. vitulorum* larvae recovered from the lungs and livers of mice which had ingested embryonated eggs, were fed to two calves in an attempt to produce infection.

Pregnant grade Jersey cows were fed eggs *per os* by means of the blow-

pipe. The cows grazed on pastures near the Laboratory and when the pastures were poor they were kept in a dry lot for feeding. Fecal examinations were made before the experiments were begun, while eggs were being fed, and following calving. The calves were taken from the dams as soon as they were dropped, to prevent contamination. These calves were kept in raised slat pens in the dairy barn or in portable pens, and fecal examinations were made every other day for at least 3 months.

In all cases where embryonated eggs were fed, the number was estimated by a dilution count based only on eggs containing motile larvae.

RESULTS

DIRECT INFECTION TRIALS.—Single doses of 1,000 to 108,000 eggs were fed to 6 calves ranging from 2 days to 6 months old. In addition, a 5-day-old calf was fed a total of 16,500 eggs in 6 doses ranging from 1,500 to 4,000 eggs per dose over a period of 16 days. Finally, approximately 10,000 eggs, which had passed unhatched through the calf fed 108,000 eggs, were in turn fed to still another calf. None of these calves exhibited any symptoms of ascariasis and no worms were found at post-mortem examinations of 3 calves killed 3, 3 and 4 weeks after having been fed eggs. Fecal examinations of these three calves were negative prior to necropsy, and the other five were negative for 6 months after feeding of eggs.

INDIRECT INFECTION TRIALS.—Two calves, 1 and 2 months old, each fed 750 *N. vitulorum* larvae recovered from experimentally infected mice, failed to become infected as determined by negative fecal examinations made every other day for 5 months.

PRENATAL INFECTION TRIALS.—Five cows ranging from 1½ to 10 years old were fed eggs at various intervals during the sixth to ninth months of pregnancy. Two of these cows (214 and 886) were also exposed during the sixth to ninth months of a second pregnancy. The data pertinent to these seven infection trials are summarized in Table 1. Fecal examinations of all cows were negative except for unhatched eggs passing through the cows during the periods of egg feeding. Of the 7 calves dropped, 2 were killed when 3 and 4 weeks old. No worms were found in either animal, and there were no discernible lesions in the lungs, livers or kidneys to indicate that larvae had migrated through these organs. No eggs were seen in fecal examinations made for 6 months in the surviving calves.

TABLE 1.—Data on pregnant cows fed eggs of *N. vitulorum*.

Cow No.	Age (Yrs.)	Date Bred	Eggs Fed In Water				Infection in Resulting Calves
			Total No.	Inclusive dates	No. Per Dose (Minimum Maximum)	Number of Doses*	
214	9	3/30/50	1,166	10/25-11/7/50	32- 198	10	0
214	10	3/27/51	815	9/4-10/16/51	120- 250	5	0
214	11	5/ 1/52	38,800	5/1/52-1/18/53	1,000- 5,200	17	0
886	4½	11/ 3/50	2,919	7/11-8/22/51	82- 207	20	0
886	5½	10/29/51	15,200	5/30/52	15,200	1	0
1120	1½	12/18/50	1,252	8/28-8/31/51	117- 570	3	0
388	7	2/21/51	815	9/2-10/16/51	120- 240	5	0
388	8	5/24/52	41,800	5/1/52-2/23/53	1,000- 5,200	17	0**
659	7	10/29/51	124,800	5/30-8/1/52	12,800-112,000	2	+** 0

*Doses evenly spaced within dates indicated. **Twins.

In addition to the above trials, eggs were fed to two cows (214 and 388) beginning at or before the time of service and continuing throughout pregnancy. These cows had been exposed to *N. vitulorum* eggs during the sixth to ninth months of two and one previous pregnancies, respectively. Cow 388 was serviced four times and, on the basis of parturition date, it appeared that conception occurred at the fourth service, the result being that cow 388 was fed eggs for 23 days prior to conception. Eggs were fed to each cow every 3 to 4 weeks until parturition, the numbers of eggs and other pertinent data being shown in Table 1. The calf dropped by cow 214 was killed 5½ weeks after birth. Fecal examinations were negative up to time of death, and neither worms nor lesions ascribable to migrating larvae were observed at postmortem. Cow 388 dropped twin calves, both of which were negative for eggs of *N. vitulorum* for 22 days; calf 1 continuing to be negative for 3 months. On the 23rd day, however, calf 2 showed a fecal egg count of 36 *N. vitulorum* per gram. The count continued to rise until 4 days later it was 296 EPG at which time a gravid female *N. vitulorum,* 14 cm. long, passed with the feces. Subsequent fecal examinations were negative, and no other worms were observed inthe droppings. However, it is possible that a male was missed, since the eggs passed were fertile.

Approximately 100 calves were reared at the Laboratory after the initiation of experiments with *N. vitulorum.* The calves were reared under the same conditions so that all had the same opportunity to acquire ascarids naturally. In view of the fact that the only calf to become infected with *N. vitulorum* was from a cow fed eggs during the complete term of pregnancy, the evidence, though admittedly presumptive, indicates that this calf was infected prenatally.

SUMMARY

Administration *per os* of infective eggs of *Neoascaris vitulorum* to 8 calves failed to produce infection. The feeding of larvae recovered from experimentally infected mice also failed to establish infection in 2 calves. Attempts to infect calves prenatally by feeding infective eggs to 5 cows during the latter third of seven gestation periods, and to one cow from the time of impregnation until parturition, were unsuccessful. Feeding infective eggs to one cow for 3 weeks prior to impregnation and continuing until parturition resulted, apparently, in the intrauterine infection of one of twin calves dropped by this cow. Twenty-three days after birth, the infected calf was passing eggs of this species and 4 days later passed a sexually mature female *N. vitulorum.*

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A New Blood Fluke, *Selachohemecus olsoni*, n.g., n. sp. (Aporocotylidae) from the Sharp-nosed Shark, *Scoliodon terra-novae**

ROBERT B. SHORT

In June 1952, five trematodes belonging to the family Aporocotylidae were recovered from the heart of a sharp-nosed shark, *Scoliodon terra-novae*, caught in Alligator Harbor, Franklin County, Florida. These flukes, all of an undescribed species, could not be assigned to an existing genus. The new genus *Selachohemecus* is therefore proposed to include them.

The family Aporocotylidae was proposed by Odhner (1912) to include the hermaphroditic, suckerless flukes believed to inhabit the vascular system of fishes. At that time the following species were known: *Aporocotyle simplex* Odhner, 1900; *Sanguinicola armata* and *S. inermis* Plehn, 1905; and *Deontacylix ovalis* Linton, 1910. Since Odhner proposed the family name, four additional genera and 14 species have been described; so that the family now contains 18 species in the following genera: *Aporocotyle* Odhner, 1900; *Sanguinicola* Plehn, 1905; *Deontacylix* Linton, 1910; *Psettarium* Goto and Ozaki, 1930; *Paradeontacylix* McIntosh, 1934; *Plehniella* Szidat, 1951 and *Cardicola* Short, 1953. Species of *Sanguinicola* and *Plehniella* have been reported from fresh water fishes only, while all other species inhabit marine hosts. In addition to the described species, is a possibly new but undescribed form found by Bazikalova (1932). This author merely lists from the intestine of the skate, *Raja radiata*, "Aporocotylidae (?) gen. sp." Aside from this questionable record, the species described herein is, to my knowledge, the only member of the family Aporocotylidae reported from an elasmobranch fish.

Although members of this family are generally considered to be blood flukes, some apparently live in the host's body cavity (e.g. *Deontacylix ovalis* and *Plehniella coelomicola;* see Manter, 1940 and Szidat, 1951). Szidat (1951) further believed that evidence indicates that some species may be capable of living in the intestinal lumen of fishes.

I wish to thank Dr. Ralph W. Yerger for identifying the shark host, Dr. Wm. Kirk for suggesting the generic name, and Mr. Allen McIntosh for reading the typescript of this paper and making valuable suggestions.

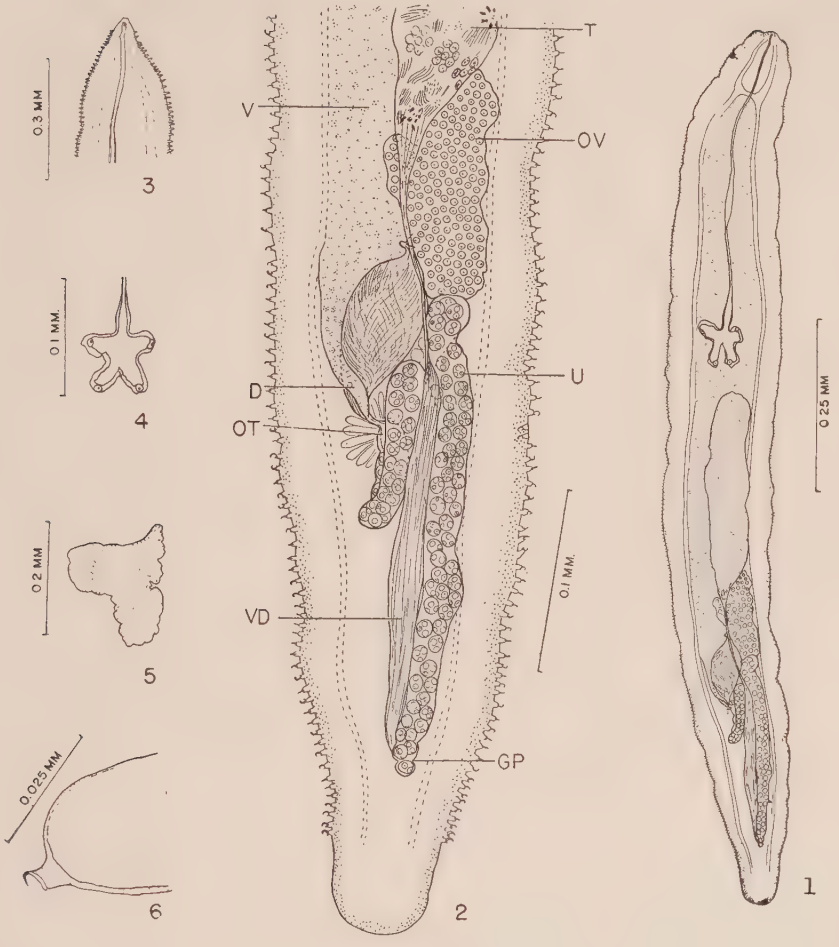
The trematodes herein considered were dissected from the shark's heart in physiological saline and were fixed in alcohol-formol-acetic solution. The shark had been dead under refrigeration for a short time before dissection and the worms were consequently in a moribund condition when fixed. Three specimens were stained and mounted whole and two were sectioned. The following description is based on a study of all five worms. Unless otherwise stated, all measurements are in millimeters; the measurements given in the specific diagnosis are from the holotype with measurements in parentheses from two paratypes (the measurements for paratype A is always given first and paratype B second).

OBSERVATIONS

Selachohemecus n.g.

GENERIC DIAGNOSIS: Aporocotylidae. Small, flat, slender trematodes; ventro-lateral body margin bearing spines. Suckers absent; pharynx absent;

*Contribution from the Zoology Department and the Oceanographic Institute (No. 22), Florida State University.



Selachohemecus olsoni

All figures were drawn with the aid of a microprojector or camera lucida.

Abbreviations: D vitelline duct; GP genital pore; OT oötype; OV ovary; T testis; U uterus; V vitellaria; VD vas deferens.

Fig. 1. Ventral view of holotype, drawn from whole mount.

Fig. 2. Posterior end of worm shown in Fig. 1, showing details of reproductive organs.

Fig. 3. Anterior end of specimen to show form when extended.

Fig. 4. Intestinal ceca, more nearly typical than those in Fig. 1 where anterior right cecum is longer than usual.

Fig. 5. Ovary in specimen more contracted than holotype, shown in Figs. 1 and 2.

Fig. 6. Cross section of lateral margin of worm showing the shape and method of attachment of spines.

esophagus long; intestine composed of four short ceca. Testicular tissue in single mass; between intestine and ovary, in approximately middle third of body. Ovary only slightly lobed, lying about one-third body length from posterior end. Uterus relatively long, entirely post-ovarial. Common genital pore, dorsal, post-ovarial, near posterior end of body. Vitellaria extensive, mostly anterior to ovary.

TYPE SPECIES: *Selachohemecus olsoni* n. sp.

Selachohemecus olsoni n.sp.

SPECIFIC DIAGNOSIS: *Selachohemecus*. Body tapering gradually toward both ends; anterior end pointed (Fig. 3) or rounded, depending on state of contraction; posterior end bluntly rounded.

Single row of spines around margin of body, continuous except at anterior and posterior ends. Spines approximately C-shaped, all similar in form and size, about 3.3 microns across arc; attached to ventro-lateral margins of body by short, cuticular projections in such a way that planes of spines are usually more or less at right angles to body plane (Fig. 6).

Mouth small, subterminal and ventral. Esophagus narrow, extending posteriad for about one-third body length, enlarging slightly before joining intestine. Intestine composed of four short ceca; two projecting laterad and usually slightly anteriad, and two posteriad and slightly laterad; anterior ceca about equal in length and slightly shorter than posterior pair, which are also of about equal length (Fig. 4). Lengths of ceca: Ant. 0.033 and 0.016 (0.016-0.035),* post. 0.031 and 0.033 (0.030-0.033).*

Testis delimited by membrane, elongate oval; lying in middle third of body, entirely posterior to intestinal ceca with longitudinal axis parallel to and to the left of midline of body; bounded anteriorly and on right by vitellaria, posteriorly by ovary and on left by nerve trunk and body parenchyma. Single vas deferens, leading from posterior tip of testis posteriad ventral to right lobe of ovary, then parallel to uterus to open dorsally through the common genital pore. Posterior two-thirds of vas deferens enlarged, containing spermatozoa and serving as seminal vesicle. No cirrus or cirrus pouch observed. Genital pore in midline, distance from posterior end of body 0.098 (0.070, 0.067).

Ovary with rather irregular margins, more or less elongate situated to left of midline of body, except for right lobe which projects slightly into right half of body; bounded anteriorly by testis, on right by vitellaria and enlargement of oviduct, on left by nerve trunk and posteriorly by uterus; length 0.130 (0.071, 0.094), width (widest part) 0.060 (0.063, 0.083).

Oviduct arising from right side of ovary posterior to right lobe, enlarging almost immediately into ovoidal chamber, then narrowing and curving mediad to enter oötype; enlarged portion filled with spermatozoa, serving as seminal receptacle, length and width 0.085 (0.071, 0.080) by 0.042 (0.041, 0.040). Mehlis' gland cells barely visible, surrounding oötype. Uterus leading from oötype posteriad for short distance, then turning sharply ventrad, and extending anteriad, looping dorsally anterior to oötype; at posterior end of ovary, turning sharply on itself and extending posteriad to union with vas deferens, immediately before discharging through genital pore. Uterus containing many eggs, thin shelled, spherical to slightly ovoidal in shape, diameter about 7.4 microns.

Vitellaria in almost solid mass, surrounding gut, to right of testis and

*The longest and shortest of the 8 anterior and 8 posterior ceca from 4 worms.

ovary, extending between lateral nerve trunks from anterior nerve commissure to level of posterior end of ovary; short vitelline duct traceable from posterior region of vitellaria to junction with oviduct immediately before oötype.

Lateral nerve trunks joined in anterior region of body by commissure dorsal to esophagus; trunks traceable from near mouth to region posterior to genital pore.

Host: *Scoliodon terra-novae* (Richardson), the sharp-nosed or Newfoundland shark.

Location: Heart.

Locality: Alligator Harbor, Franklin Co., Fla.

Type: Holotype is deposited in the Helminthological Collection of the U. S. National Museum, No. 37408. Paratypes in author's collection.

The species is named in honor of Dr. F. C. W. Olson, of the Oceanographic Institute of Florida State University, who collected the host and who has always been very cooperative in supplying fishes for dissection.

Discussion

Although spines are common on members of the family Aporocotylidae the C-shape of the spines on *Selachohemecus olsoni* apparently are unique among known species of this group. In fixed and mounted specimens, many of the cuticular projections bearing spines are at right angles to the plane of the body; however, many spines are oriented with the points curving ventromesiad as shown in figure 6, and it is likely that in life this is their normal position, a position which seems well adapted for clinging to the walls of the vascular system of the host.

The right lobe of the ovary, mentioned in the specific diagnosis as projecting into the right half of the body, is more distinct in specimens not extended as greatly as the type, shown in figures 1 and 2 (see Fig. 5).

A study of the literature on the family Aporocotylidae reveals that the following characters are of generic importance: 1) position (pre- or post-ovarial) and length of uterus, 2) separate or common genital pores, 3) position of genital pore (pre- or post-ovarial), 4) number, arrangement, shape and possibly nature (reticulate or not) of testes, 5) shape of intestine, and 6) shape of ovary (degree and character of lobation). Szidat (1951) also considers the character and size of the cirrus pouch in *Plehniella* as of generic significance.

On the basis of these criteria, erection of the genus *Selachohemecus* is justified. Placing the present species in a new genus is further supported by the fact that it is from an elasmobranch fish while all other aporocotylids are from bony fishes.

Selachohemecus is similar to *Psettarium, Paradeontacylix* and *Cardicola* in having a relatively long post-ovarial uterus with post-ovarial genital pores. *Selachohemecus,* however, has a common male and female genital pore, whereas the pores in the other three genera are separate. *Selachohemecus* further differs from all three genera in its much shorter intestinal ceca, and from *Paradeontacylix,* in addition, by having a single testis.

Deontacylix can be separated from *Selachohemecus* by its pre-ovarial uterine coils, separate genital pores, and a more or less H-shaped testis.

Aporocotyle is the only genus similar to *Selachohemecus* in having common male and female pores. The two genera differ in *Aporocotyle's* pre-ovarial uterus and genital pore, much longer intestinal ceca and many testes.

Sanguinicola and *Plehniella* both resemble *Selachohemecus* in their short

intestinal ceca, the gut of *Selachohemecus* being especially like that of most species of *Sanguinicola*. The gut in *Plehniella* has six ceca in contrast to four in *Selachohemecus*. Both *Sanguinicola* and *Plehniella* are from freshwater fishes, however, and can be separated morphologically from *Selachohemecus* by their very short uteri, separate genital pores, bolobed ovaries, and division of testicular tissue into several follicles.

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Correlation of Growth Rate and Severity of Cecal Lesions In Chicks Experimentally Infected with Cecal Coccidiosis

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For a number of years it has been the practice at this station, when conducting studies on the effects of drug and other treatment of cecal coccidiosis due to *Eimeria tenella*, to classify the cecal condition found on post-mortem examination. Each chick is assigned to one of four grades, numbered 0, 1, 2, and 3. Where no sign of injury is discernible, they are classified as grade 0; barely recognizable lesions, grade 1; cecal walls thickened and small to medium cores, grade 2; and very thick walls, large cores, or much blood, grade 3.

More recently a mathematical formula, as used by Waletzky, Hughes, and Brandt (1949), has been applied to these findings, and a value, termed "average grade of lesions," derived from the application of this formula, has been used as an aid to the evaluation of the effect of treatment. The average grade of lesions is obtained by multiplying the number of chicks falling within each grade by the corresponding grade designation, totalling the products of these calculations, and dividing the sum thus obtained by the total number of chicks in the group. This may be expressed by the equation AGL $=$

$$\frac{X_1 + 2X_2 + 3X_3}{X_0 + X_1 + X_2 + X_3}$$

In order to determine how much, if any, correlation existed between the severity of the disease as indicated by the condition of the ceca and as indicated by the rate of growth of the infected chicks, a critical analysis of the relevant data of several experiments was made. The average grade of lesions discussed above is not germane to the present thesis, but does have a pertinency to its extension.

Material, Methods and Procedure

Four experiments were selected for analysis. They were chosen because in them each category of cecal condition was represented by a sufficient number of chicks to make a comparative analysis possible. The drug used and

its over-all effect on the severity of the infection were not considered as criteria in the selection of experiments for analysis.

In each experiment the surviving chicks were grouped according to grade of cecal lesions. All infected chicks of grade 0, for example, were grouped together without regard to period of treatment. The weight gain made by each chick was recorded and an average gain for each group obtained. The average gains made by the different groups were then compared with each other and with the average gain made by the uninoculated controls over the same period, and were tested for statistical significance.

RESULTS

The average gain per bird made by the uninoculated controls proved, with one exception (experiment 2), to be well above that made by any of the inoculated groups (see Table 1). The difference was statistically significant

TABLE 1.—Average gain per bird (grams)

Category	Experiment number			
	1	2	3	4
U U C*	143.1	119.1	170.4	72.4
Gr. 0	136.6	109.5	156.3	61.2
Gr. 1	135.1	121.6	141.9	55.9
Gr. 2	97.2	91.0	119.3	50.9
Gr. 3	41.2	59.4	86.4	45.3

*Uninoculated, untreated controls.

in every case when compared with the grade 2 and grade 3 groups, but was not so in 2 experiments (1 and 2) when compared with the grade 1 group or in 3 experiments (1, 2, and 3) when compared with the grade 0 group. The exception to the consistently better growth made by the uninoculated controls, mentioned above, which occurred in experiment 2, where the grade 1 chicks exceeded the controls in average gain per bird, cannot be given too much weight, since there were only 5 chicks that fell within the grade 1 category in that experiment and one of these made a much greater gain than the group average; the difference, in any case, had no statistical significance.

Grade 0 and grade 1 were very close together in average gains, although the grade 0 chicks showed greater gains than did those of grade 1 in 2 experiments (3 and 4). They were practically identical in 1 experiment (1), and in another experiment (2), the exception discussed above, the grade 1 chicks made the greater average gain. The difference in growth between grade 0 and grade 1 was statistically significant in only 1 experiment (3), but it was an experiment in which the greater growth was made by the grade 0 chicks.

The grade 2 group in no case equalled the weight gains made by the grade 0 or grade 1 groups, but in every case exceeded that made by the grade 3 group. The statistical significance of these defferences was high, with the exception of experiment 4, where there was no significant difference between grade 2 and either grade 1 or grade 3.

The grade 3 group made the poorest growth in every case, and the statistical significance was very high, with the one exception mentioned above.

The difference in weight gains between the various categories is illustrated in Table 2, where the percentage of gain of each group is set forth. Percentages are based on the gains made by the uninoculated controls, these being assigned a value of 100 percent. It will be seen from this tabulation, perhaps more clearly than in Table 1, that there was, with the single exception

in the case of the grade 1 group in experiment 2, a consistent drop, grade by grade, from 0 through 3.

TABLE 2.—Average gain per bird expressed as percent of gain of U U C

Category	Experiment number			
	1	2	3	4
U U C	100.0	100.0	100.0	100.0
Gr. 0	95.5	91.9	91.7	84.5
Gr. 1	94.4	102.1	83.3	77.2
Gr. 2	67.9	76.4	70.0	70.3
Gr. 3	28.8	49.9	50.7	62.6

In Table 3 the minimum and maximum gains (i.e., poorest bird and best bird) in each group are given. It will be noted that the minimum gains made by grades 2 and 3 are well below those in the other groups, while the minimum gains made by grades 0 and 1 compare fairly well, in most cases, with the control minimums. On the other hand, the maximum gain in the grade 3 group was invariably the poorest, whereas it was fairly uniform among the other groups.

TABLE 3.—Minimum and maximum gains (grams)

Category	Minimum gain				Maximum gain			
	Experiment number				Experiment number			
	1	2	3	4	1	2	3	4
U U C	85	95	118	52	206	143	231	95
Gr. 0	97	93	142	40	187	124	179	83
Gr. 1	76	108	92	27	194	140	190	82
Gr. 2	—6	9	54	8	193	135	238	90
Gr. 3	11	14	56	6	97	98	114	74

Conclusions and Summary

There appeared to be an inverse correlation between growth rate and severity of cecal lesions in chicks having *Eimeria tenella* infections in the experiments recorded in this paper. It is perhaps worthy of note that in other experiments that were analyzed, but were not used because of an insufficient number of chicks in one or more categories, the results in every case confirmed the findings set forth here. Discrepancies in the case of individual chicks may very well arise from the fact that post-mortem examination is delayed until some of the chicks have partly recovered from the effects of the disease. This seems particularly evident in the case of the grade 2 groups, where the range from maximum to minimum is very wide. Probably a certain number of birds in each group would have been classified 1 or 2 grades lower had the post-mortem been conducted earlier in the course of the disease. After all, grade of lesions at post-mortem represents just one moment in a changing picture.

Scatter diagrams of these data do not readily suggest any elementary statistical expression of the relationship between the factors involved. On the whole, however, the results of this analysis indicate that the system of grading and weighing the severity of cecal lesions is basically sound and in full accord with other observed data.

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Germinal Development in the Sporocysts of the Blood Flukes of Turtles*

W. W. CORT, D. J. AMEEL, AND ANNE VAN DER WOUDE

The turtle blood flukes of the family Spirorchiidae show a very clear relationship to the true schistosomes in the structure of their larval stages and in the pattern of their life cycles. The fact that the sexes are not separate in the adult stage has been taken as evidence that they are more primitive than the Schistosomatidae. Therefore, it seemed of interest to study the development of the germinal material in the mother and daughter sporocysts of representatives of the Spirorchiidae for comparison with that in the schistosomes. During the summers of 1951 and 1952 at the University of Michigan Biological Station we obtained developmental stages of mother and daughter sporocysts of a spirorchiid in experimental infections. The only turtle blood fluke that had been previously identified in the immediate vicinity of the Biological Station was *Cercaria elephantis* Cort, 1917 which had been frequently collected in the species of *Helisoma* from the shore of Douglas Lake and from the streams and ponds of the area. *C. wardi* Miller 1923, another spirorchiid cercaria which can be easily distinguished from *C. elephantis,* has only been found at Twin Lakes, about 40 miles from the Station. Therefore, we took it for granted when these studies were started that we were working only with *C. elephantis.*

To obtain miracidia for infection experiments, eggs were isolated from the feces of naturally infected specimens of *Chysemys picta* collected from places where *C. elephantis* was known to be present. Several different infected turtles were used each summer. In 1951 we did not autopsy any of the turtles from which the eggs were obtained since they had been loaned to us from collections that were being used for another investigation. However, in the summer of 1952 we autopsied two of the infected turtles. In the one, from which the largest number of eggs had been obtained, we found five adult blood flukes and in the other only one. These adults belonged to two different species which have not yet been identified. This was not a complete surprise because we had found in the summer of 1952 two different types of daughter sporocysts. The commonest of these, which was the only one found in 1951, had about 25 to 35 cercarial embryos in the daughter sporocysts at the stages just before or just after their migration from the mother. The other type, of which only four infections were found, had from 50 to 100 cercarial embryos in the daughter sporocysts at these stages. After this finding we kept as many of the infected snails as could be spared in the hope that mature cercariae would develop. From three of them cercariae finally escaped and in another at autopsy mature active cercariae were present. Three of these infections were identified as *C. elephantis,* which was found to be the form with the smaller number of cercarial embryos in the daughter sporocysts. The cercariae that had developed in the other snail was a different species which was easily distinguished from *C. elephantis* by its much larger size and by certain clear-cut differences in structure. This confirmed the earlier conclusion that two species were present in our experimental infections. Further studies, including infection experiments, will be necessary to determine which of the two species of adult blood flukes found in the turtles used in the infection experiments of

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the summer of 1952 belongs to *C. elephantis* and which to the larger cercaria. Wall (1941) identified as *C. elephantis* the cercaria of a species of *Spirorchis* that he studied in southern Michigan. However, Wall's cercaria is distinctly different from *C. elephantis,* and his adults are different from either of the two species we obtained from our experimental turtles. Further studies are under way in an attempt to clear up the confusion in the identification of the adults and cercariae of the Spirorchiidae in the Douglas Lake region.

A preliminary account of the investigations that are reported in this paper was given in a previous publication (Cort, Ameel and Van der Woude, 1954a).

Materials and Methods

As already stated *Spirorchis* eggs were obtained from the feces of naturally infected turtles of the species *Chrysemys picta.* These turtles were captured in two streams near the Biological Station, Fountenalis Run and Niger Creek. They were kept in large jars in the laboratory and their feces were examined for eggs with direct smears after sedimentation. The eggs were passed irregularly by the infected turtles and were found in largest numbers in mucous pellets. We found no satisfactory way of isolating the eggs except by teasing them from the pellets with sharp needles and picking them out with fine pipettes. This proved to be a very tedious procedure since for satisfactory development and hatching the eggs had to be completely separated from all fecal debris. The eggs that were isolated were kept in small Stender dishes in which the water was frequently changed. These dishes were frequently examined for free-swimming miracidia, which were found in largest numbers in the early morning.

The snails used in the infection experiments were laboratory raised juveniles of *Helisoma trivolvis.* Most of the eggs from which they hatched were obtained by scraping egg masses from sticks picked up in a stream where there were large numbers of adults of this species. The juvenile snails in the laboratory cultures became infected with Chaetogasters which were seen to feed on miracidia. Therefore, in the later infection experiments an attempt was made to rid the snails of the Chaetogasters by mechanical manipulation with sharp needles before exposing them to the miracidia. The standard procedure was to put four miracidia with each snail in a small amount of water and to watch for penetration. After exposure to the miracidia, the snails were placed in larger containers. Few of the experimental snails died, but not as large a number as we expected were infected. In the summer of 1951, 13 out of 55 snails that were exposed to miracidia showed infection on examination. In the following summer we at first obtained only 12 per cent infection; but later by removing the Chaetogasters and with more careful handling the percentage was increased considerably. In this summer 38 or 29 per cent of the 133 exposed snails were found to be infected.

Methods of study of the germinal development in the sporocysts were the same as those described in recent papers of this series. Most of the observations were made on living material, but sections were available for the study of most of the stages.

Studies on Germinal Development

Miracidium—mother sporocyst stage. Miracidia from the cultures that were used in the experimental infections were studied in the summer of 1951 to determine the number and location of the germinal cells. The arrangement of the germinal cells in the miracidium is shown in figure 1. They are rather

large cells which are in contact with each other forming a group in the body cavity in the posterior half of the body of the miracidium. The number of germinal cells in counts made on seven miracidia ranged from 9 to 14 with an average of 12.

The mother sporocysts in the experimentally infected snails were found in the mantle. Most of the smaller ones were freed when the mantle was teased apart with sharp needles. Larger mothers were attached tightly to the tissues of the mantle and were very difficult to isolate without breaking. No attempt was made to find mother sporocysts in infections under three days of age, so we have no observations on the metamorphosis of the miracidia into mother sporocysts.

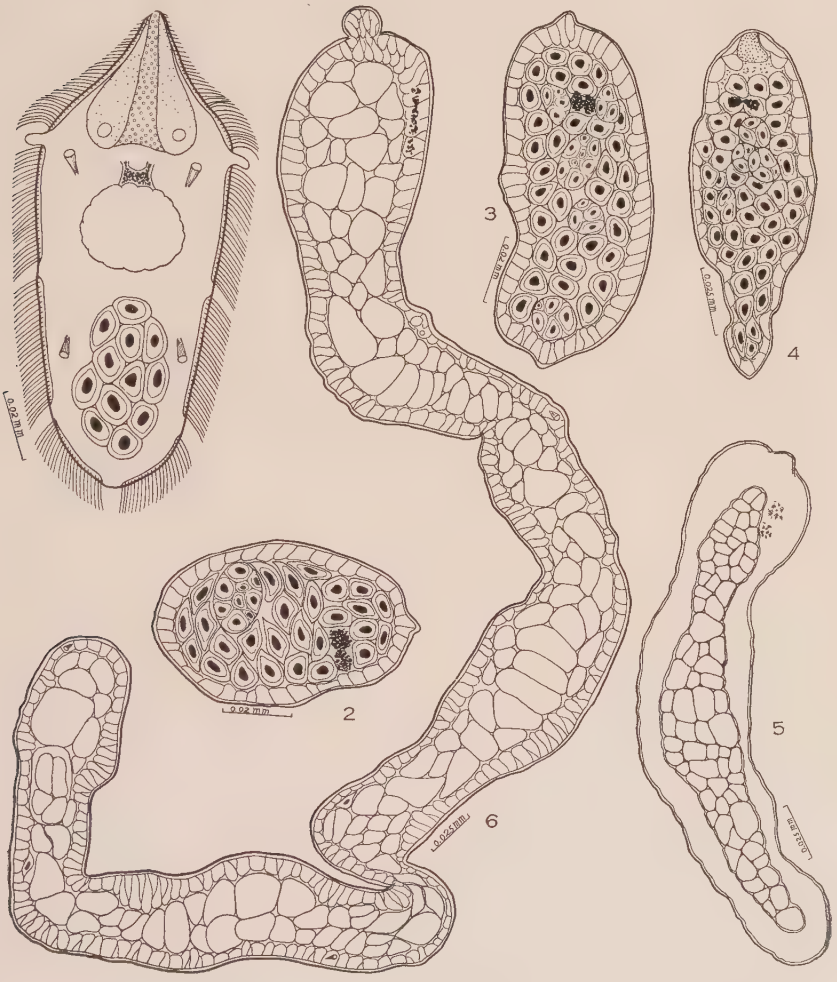

Plate 1. Miracidium and mother sporocysts of spirorchiids.

Fig. 1. Miracidium showing the arrangement of the germinal cells.
Figs. 2, 3, 4. Three day old mother sporocysts.
Fig. 5. Mother sporocyst, 0.24 by 0.05 mm, from a five day old infection.
Fig. 6. Mother sporocyst, 0.98 by 0.08 mm, from a six day old infection.

Three day old mother sporocysts are shown in figures 2, 3 and 4. By this time the metamorphosis from the miracidium was almost completed, since the only larval characters that remained were remnants of the eyespots and of the glandular complex at the anterior end. The sporocyst wall was quite thick and the anterior end was marked by the location of the eyespots and a slight projection. The smallest of the three day old mother sporocysts, which measured 0.072 by 0.046 mm (Fig. 2), was evidently somewhat contracted with its germinal cells rather elongate and crowded together. Some of them appeared to have cytoplasmic projections. It had between 25 and 30 germinal elements in the body cavity, at least one of which was an embryo of about eight cells. It is probable that some two to three cell embryos were also present, because it is very difficult in such specimens to distinguish them from the single germinal cells. Figure 3 shows a slightly larger three day old mother sporocyst, which measured 0.096 by 0.042 mm. Its body cavity was not quite as crowded and the germinal cells appeared more rounded than in the mother sporocyst shown in figure 2. More than 35 germinal elements were counted in it, as least six of which were small embryos. Figure 4 shows the largest of the three day old mothers, which measured 0.127 by 0.043. Its body cavity contained between 40 and 50 germinal elements, at least five of which were small embryos.

The five day old mother sporocyst shown in figure 5 measured 0.24 by 0.05 mm. Remnants of the eyespots were still present, and there was a slight papilla-like projection at the anterior end. The wall was very thick and the germinal material crowded, making it difficult to determine which of the germinal elements were embryos and which germinal cells. About 70 germinal elements were counted, more than half of which were considered to be embryos. It seems probable that many germinal cells attached to the wall could not be seen and were therefore not included in this count. The largest daughter sporocyst embryos in this mother measured only about 0.020 to 0.025 mm in diameter.

We were able to obtain only a few sections of the early stages of the mother sporocysts. These showed the rather thick wall and the differences between the cells fo the body wall and germinal cells. Some of the germinal cells appeared to be in the wall as shown in figure 12 which is a cross section near the posterior end of a 4 to 5 day old mother sporocyst. The sections of this mother sporocyst showed fibrous projections from the germinal cells and gave the impression that both the germinal cells and small embryos were held in a network in the body cavity. The study of the sections also indicated that the number of germinal cells at this stage that were in close contact with the wall was considerably greater than could be seen in the examination of living material.

The mother sporocyst from a 6-day old infection that is shown in figure 6 measured 0.98 by 0.08 mm. A trace of the pigment of the eyespots could still be seen near its anterior end. While the width varied considerably there were no constricted places without embryos in them. The number of germinal elements that were actually counted was 110, most of which appeared to be small embryos. There were probably many more germinal cells which could not be seen because of the thickness of the wall. The largest of the embryos were still quite small, about 0.030 to 0.040 mm in diameter, and all were still in the "germ-ball" stage.

An unbroken mother sporocyst about 3 mm in length was obtained from an eleven day old infection. It was very irregularly twisted, and varied greatly in width. The widest portions that were filled with embryos were about 0.10

to 0.12 mm in width, while in certain regions the walls were so contracted that their inner surfaces were in contact. A few germinal cells attached to the wall were seen throughout the length of this mother sporocyst and probably many more were present. About 300 embryos of different sizes were actually counted, most of which were under 0.030 mm in diameter. Only a small proportion were of larger size, the largest being 0.080 by 0.060 mm. The largest embryos although somewhat elongate did not show characters that would have made them recognizable as daughter sporocysts.

In these early stages of mother sporocysts that have just been described there was, even in the limited series of specimens that we were able to study, a rather surprising variation in size in infections of the same age. For example,

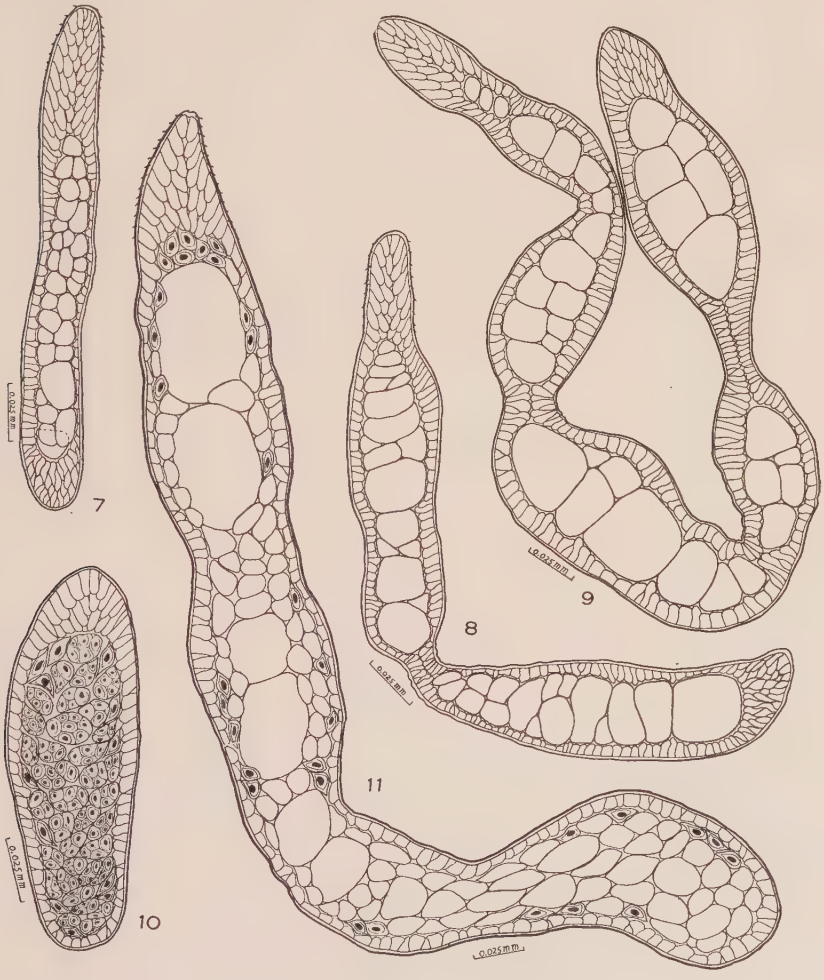

Plate 2. Spirorchiid daughter sporocysts.

Fig. 7. Small daughter sporocyst of *C. elephantis*, 0.22 by 0.02 mm.
Fig. 8. Daughter sporocyst of *C. elephantis*, 0.35 by 0.04 mm.
Fig. 9. Daughter sporocyst of *C. elephantis*, 0.70 by 0.05 mm.
Figs. 10 and 11. Daughter sporocysts of spirorchiid with large cercaria.

a six-day old mother measured 0.97 by 0.08 mm, while one 7 days old was only 0.53 by 0.06 mm. One mother 10 to 12 days old measured only 0.96 by 0.06 mm, while another the same age had a length of about 3 mm and a width varying from 0.06 to 0.012 mm. Due to scarcity of material mother sporocysts from 12 to 19 days of age were not studied.

Some data on older mother sporocysts were obtained during the summer of 1952 from the examination of experimental infections that were from 19 to 40 days old. All of these infections were identified as *C. elephantis* since they contained daughter sporocysts in which from 25 to 35 cercarial embryos could be counted. Pieces of single mother sporocyst were isolated from a snail that had been exposed to miracidia 20 or 21 days previously. Measurement of the pieces showed that this mother was about 6 mm long. It had several constricted places which varied in width from 0.03 to 0.04 mm. The wider portions that were crowded with embryos varied from 0.06 to 0.10 mm. Germinal cells and very small embryos could be seen attached to the wall when the constricted parts were examined with the high power. The anterior end of this mother was greatly attenuated for a distance of about 0.25 mm. In it the birth pore and birth canal could be clearly seen. About 125 daughter sporocyst embryos in various stages of development were counted in the pieces of this mother, not including the germinal cells and very small embryos attached to the wall. A number of these daughter embryos were from 0.2 to 0.3 mm in length, were active, and appeared to be about ready to escape. The digestive gland of this snail was teased apart and 178 daughter sporocysts that had escaped from this mother were counted in it. Pieces of the mother sporocysts were found in several still immature older infections 34 to 40 days old. In these the mothers appeared to be exhausted since most of the pieces were empty and what few embryos were present appeared to be dead. In these infections also free daughter sporocysts in the migrating stages were not found.

Sections of mother sporocysts which contained some active daughter sporocysts gave some additional information on the structure of the mother sporocysts. In them the wall was thinner than in the earlier stages, and the nuclei of its cells were flattened. Germinal cells and very small embryos which consisted of a few cells could be seen attached to the wall by fibrous cytoplasmic projections. In some portions of mothers at this stage there were no active daughters and the larger embryos as well as the germinal cells and small embryos attached closely to the wall appeared to be held in a fibrous network. A section of such a portion of a 24 to 25 day old mother sporocyst is shown in figure 13. In other parts of this mother there were active daughters and some were already in the snail's digestive gland. The part shown in the drawing contained daughter sporocyst embryos of a variety of sizes ranging from single germinal cells to those considerably over 0.1 mm in length which contained a number of cercarial embryos. The smallest embryos were attached closely to the wall, while the others were held in position by the network of fibers. Sections of mothers at this and somewhat later stages showed that germinal cells and small embryos attached to the wall were still numerous in mothers from which large numbers of daughters had escaped.

DAUGHTER SPOROCYSTS OF *C. elephantis*. As noted in the previous section the germinal cells that are to form the daughter sporocysts usually appear to be attached to the wall of the mothers by projections of their cytoplasm. Small embryos are also attached to the wall as shown along the upper left-hand side of figure 13. In some of them germinal cells can be distinguished from somatic cells. A slightly older daughter sporocyst embryo in the "germ ball" stage is shown on the other side of this drawing in the upper right-hand

corner. In it the germinal can be clearly distinguished from the somatic cells surrounding them which will form the inner layer of the wall of the daughter sporocyst.

In elongate daughter sporocyst embryos of *C. elephantis* slightly under 0.1 mm in length the body cavity containing the germinal material is clearly defined, and the inner layer of its wall forms a plug at the anterior end. The smallest daughter sporocyst embryo at this stage that was studied measured 0.08 mm by 0.05 mm. In it the thin outer layer of the body wall with its flattened cells could be made out. The anterior plug of body wall cells extended for about two-fifths of the body length. The body cavity contained 12 germinal cells and there were two small cercarial embryos at its posterior end. The germinal cells were attached to the body wall and to each other by cytoplasmic processes. The structure of the cells of the body wall and the germinal elements at this stage can be seen in a longitudinal section of a slightly larger daughter sporocyst embryo in the lower part of figure 13. This section shows clearly that the anterior plug is simply a mass of the cells of the inner layer of the body wall. The germinal cells in the body cavity can be easily distinguished

Plate 3. Sections of spirorchiid mother and daughter sporocysts.

Fig. 12. Cross section near the posterior end of a 4 to 5 day old mother sporocyst.

Fig. 13. Section of a portion of a 24 to 25 day old mother sporocyst.

Fig. 14. Longitudinal section of a daughter sporocyst of *C. elephantis*.

Fig. 15. Cross section of a daughter sporocyst of *C. elephantis*.

from the cells of the plug and the inner layer of the body wall. In the cercarial embryos in the body cavity the germinal can be distinguished from the somatic cells. The spines on the anterior tip which are characteristic of the immature daughter sporocysts of spirorchiids and schistosomes can be seen even at this early stage.

Figure 14 is a longitudinal section of a young free daughter sporocyst of *C. elephantis* from a 24 to 25 day old infection, which measured about 0.26 by 0.05 mm. In sections of this daughter the spines on the anterior end could be seen and the anterior plug of body wall cells extended for about one-fourth of the length. Numerous germinal cells and cercarial embryos composed of a few cells were attached to the wall of the body cavity. The body cavity contained numerous cercarial embryos, which were held in a fibrous network. In them the germinal could be distinguished from the somatic cells.

Figure 15 is a cross-section of a daughter sporocyst which was in the digestive gland of a 21-day old infection. In this section the nuclei of the cells of the thick body wall can be easily distinguished from the germinal cells attached to the wall. These and the embryos that are present in the body cavity are held in a fibrous network.

Studies of living specimens of young daughter sporocysts of *C. elephantis* were made in the latter part of the summer of 1951 from infections that were from 40 to 45 days old. They were evidently retarded in development by the unusually cool weather that prevailed in the Douglas Lake region during August of that year since in none of them were cercarial embryos present that showed a division into body and tail. The daughter sporocysts in these infections appeared to be normal and did not differ in structure from those that were studied from the more rapidly developing infections of the summer of 1952. The smallest daughters found in the 1951 series were about 0.2 mm in length and the largest were from 1.5 to 2 mm. Figure 7 shows one of the smallest of these daughter sporocysts. Its size was 0.22 by 0.02 mm, and about 30 embryos could be counted in its body cavity, the largest of which were only 0.020 to 0.025 mm in diameter. Another of the smallest daughters measured 0.20 by 0.03 mm and contained about 30 cercarial embryos, which were about the same size as those shown in figure 7. In daughter sporocysts of this size, the walls were thick and only a few germinal cells could be seen. A somewhat larger daughter sporocyst, 0.35 by 0.04 mm, is shown in figure 8. About 35 cercarial embryos could be counted in it, the largest of which at the posterior end had a diameter of 0.030 mm.

The largest daughter sporocysts in this series of infections were usually more irregular in shape, having constricted places separating wider portions in which the embryos were crowded. An example of this is shown in figure 9 which depicts a daughter 0.70 by 0.05 mm with four constricted places. Thirty-five embryos were counted in this daughter, the largest of which had a diameter of about 0.04 mm. Such constricted regions became more pronounced in some of the larger daughters. In them a few germinal cells could usually be seen at the anterior end of the body cavity or along the wall. The anterior plug was still quite prominent although its size in relation to the total length was considerably reduced. There was little if any increase in the number of embryos that could be counted since in most of the largest sporocysts the number was between 30 and 40. In a daughter 1.1 by 0.07 mm there were three constricted places and 35 embryos were counted, the largest of which was 0.12 by 0.065 mm. The largest daughter that was studied from the 1951 series was 2 mm long by about 0.1 mm wide in the unconstricted portions. The number of cercarial embryos in it was between 30 and 35 of which the largest

was 0.12 by 0.07 mm and showed no division into body and tail. In it, as in all the largest daughter sporocysts examined alive, only a few germinal cells and small embryos attached to the wall could be seen. However, in sections of daughters at this stage numerous germinal cells and very small embryos could always be seen attached to the wall. This indicated that we were unable to see in living specimens most of the small germinal elements attached to the body wall because of its thickness.

In the summer of 1952 a number of experimental infections of *C. elephantis* were examined in which many of the daughter sporocysts were larger and further along in development than any of those seen in the 1951 series. Most striking was the large size of the largest cercarial embryos in relation to the size of the daughter sporocysts. These almost mature daughter sporocysts varied greatly in the number and extent of the constricted portions. One sporocyst 2.8 mm in length had a width varying from 0.04 to 0.10 mm, with only one constricted portion. It contained almost 40 cercarial embryos of which 16 had well-developed bodies and tails. None were yet active. The largest cercarial embryos had a total length of 0.65 mm, the body being 0.32 mm long and the tail stem 0.20 mm. A few of the larger daughters in this infection, which was from 23 to 25 days old, were from 1.5 to 2.5 mm long and contained from 2 to 8 large cercarial embryos with eyespots and a few more than showed division into body and tail.

In the three mature experimental infections of *C. elephantis* the largest daughter sporocysts were from 3 to 4 mm in length. In them, the cercarial embryos, most of which were well-developed and a few actively moving, crowded the body cavity. One daughter 4 mm in length contained 27 well-developed cercarial embryos with eyespots. They crowded the body cavity and very few other embryos could be seen. Another daughter 3 mm in length had 28 well-developed cercarial embryos with eyespots, most of which were active, besides a few small embryos squeezed in between them. The average length of the cercariae of *C. elephantis* is well over 1 mm including body and tail, and the embryos in which eyespots can be seen are nearly as large. Therefore, it is not surprising that mature daughter sporocysts are crowded with cercarial embryos by the time that the first fully developed cercariae are ready to escape.

In the summer of 1952, three infections were found in which the daughter sporocysts contained much larger numbers of cercarial embryos than those of *C. elephantis*. It can be suggested that these sporocysts belong to the species with the larger cercariae. Figures 10 and 11 represent sporocysts of this type. In addition to having larger numbers of cercarial embryos than the daughters of *C. elephantis,* the sporocyst wall is thinner which made it possible to see larger numbers of germinal cells.

DISCUSSION

The pattern of germinal development in the Spirorchiidae is of special interest because of their relationship to the schistosomes. It is characterized in both the mother and daughter sporocysts by the division of some of the germinal cells over a long period of time. Others, however, begin very early to develop into embryos. No germinal masses are present. Multiplication of germinal cells has already begun in the miracidium and proceeds rapidly in the mother sporocysts, since in three day old mothers there are already 25 to 40 germinal elements some of which have already developed into daughter sporocyst embryos. The mother sporocysts grow rapidly in size to give space for the

developing daughters. However, the daughter sporocyst embryos increase very slowly in size during the first half of the growth of the mothers and then grow very rapidly to the migrating stage. While the evidence is rather meager it indicates that daughter sporocysts at favorable temperatures first begin to escape from the mothers in less than three weeks and that all that will be produced have escaped in less than 5 weeks. In mother sporocysts containing numerous active daughters there are present many smaller embryos as well as numerous germinal cells and very small embryos attached to the walls. However, in infections containing well-developed cercarial embryos only degenerate pieces of the mother sporocysts were ever found. While it was impossible to make accurate measurements because we were not able to isolate the older mother sporocysts without breaking we have evidence that they grow to a length of at least 6 mm. The mothers produce more than 300 daughters which is more than enough to fill the digestive gland of even the largest snail intermediate hosts. After the escape of the daughters from the mother they appear to develop very rapidly in the digestive gland of the snail since in infections that contained fully developed cercariae practically all of the daughters were of large size and contained well-developed cercarial embryos.

Sections of young mother sporocysts containing only small embryos showed that germinal cells and the smallest embryos were held in a fibrous network in the body cavity of the mother. As the daughter sporocyst embryos became larger and active they break down this network and move freely in the body cavity of the mother.

As already mentioned the germinal cells and smallest embryos in the mother sporocysts are attached to the wall by cytoplasmic projections. As the daughter sporocyst embryos develop to the "germ ball" stage they show a thin outer layer of the body wall and an inner layer of somatic cells surrounding the germinal cells in the primitive body cavity. As they begin to elongate the body cavity becomes clearly defined and the somatic cells form a plug at the anterior end. In these early stages of development the small spines at the anterior tip can be clearly seen. Sections of very young elongate daughter sporocyst embryos show that germinal cells and the smallest embryos are attached to the wall by fibrous strands and the larger embryos in the lumen of the body cavity are held in a fibrous network. Germinal cells begin to develop into cercarial embryos at a very early stage and they develop slowly until after the daughter sporocysts have escaped from the mother and established themselves in the digestive gland of the snail host; not until the daughter sporocysts reach considerable size do the cercarial embryos show division into body and tail. Germinal cells and small embryos attached to the wall are still present in large daughter sporocysts which contain well-developed cercariae. The fully developed cercariae are very large compared with the size of the daughter sporocysts and crowd the body cavity.

While most of the observations recorded above were made on *C. elephantis* four of the experimental infections of the summer of 1952 were of another species, the cercaria of which was very much larger. It is of special significance that this species can be distinguished from *C. elephantis* by the larger number of cercarial embryos in the immature daughter sporocysts. It seems very probable that the type of germinal development in the sporocysts of these two species is characteristic of the family Spirorchiidae.

Pieper (1953) has recently investigated the germ cell cycle of a representative of the Spirorchiidae, *Spirorchis artericola*. Her studies, which were made on sections of fixed material, showed that the theory of germinal lineage with multiplication by polyembryony of the original zygote (fertilized ovum)

applies to this form. In the multiplication of the germinal cells in the sporocysts she found no suggestion of maturation phenomena or polar body formation and no indication that germinal cells were produced from somatic cells of the body wall. The germinal cells divided repeatedly and developed directly into embryos of daughter sporocysts and cercariae without the formation of germinal masess, and in the cercarial embryos they formed the genital primordium. The exact number of chromosomes in the germinal cells was not determined. However, the author stated that the comparison of the metaphase plate of dividing germinal cells in young sporocysts with the dividing somatic cells in developing cercariae showed similarity in the number and position of the chromosomes. Our studies confirm those of Pieper and since they were made for the most part on living material have made it possible to show the structure of the different stages in the development of the mother and daughter sporocysts.

A comparison of the stages of development of the sporocysts of the spirorchiids with those of the schistosomes shows a close resemblance and the same pattern of germinal development. Their mother sporocysts are very similar to those obtained in experimental infections of *T. stagnicolae* (Cort, Ameel and Van der Woude, 1954a and b), but differ from the mothers of *Schistosomatium douthitti* (Cort, Ameel and Olivier, 1944) which grow into inflated sacs with very thin walls in which the development of the daughters is almost synchronous. This stage in the spirorchiids also differs in structure and arrangement of the germinal material from the mother sporocysts of *T. stagnicolae* found by Cort and Olivier (1943) in natural infections in adult snails. The daughter sporocysts of the spirorchiids are very much like those of the schistosomes both in the structure and the pattern of germinal development, particularly in the early stages of their development. In very small daughter sporocyst embryos of *C. elephantis* the size of the plug of body wall cells at the anterior end resembles that in the same stage of *S. douthitti* (cf. fig. 13 with fig. 10 of Cort, Ameel and Van der Woude, 1953). In both the spirorchiids and schistosomes cercarial embryos start to develop very early, but grow very slowly until the daughter sporocysts have grown to considerable size in the digestive gland of the snail host. In the daughter sporocysts of *C. elephantis* the number of cercarial embryos produced seems to be less than in the schistosome species. This species also develops much more slowly in the snail intermediate host than *T. stagnicolae*, being in this respect like *S. douthitti*. In both the spirorchiids and the schistosomes the production of cercariae is limited to a much shorter period than in the strigeoids. In these groups by the time the first cercariae are ready to escape all the daughter sporocysts that can develop have reached full size, and contain large cercarial embryos.

Abstract Summary

Studies were made of the germinal development in the mother and daughter sporocysts of *C. elephantis* in experimental infections of the snail intermediate host. A few infections of another species with a much larger cercaria also developed in the experimentally infected snails. Very rapid multiplication of the germinal cells takes place in the early stages of the mother sporocysts and some of them have developed into embryos in three day old mothers only about 0.1 mm. long. The mother sporocysts grow rapidly in size and those about 1 mm in length contain over 100 germinal elements most of which are embryos. By the time that mother sporocysts are 3 mm in length in 10 to 12

day old infections the number of germinal elements has increased to over 300, but the largest daughter sporocyst embryos are small "germ balls" only about 0.03 mm in diameter. After this, the embryos grow very rapidly and daughter sporocysts begin to migrate to the digestive gland of the snail about 21 days after infection. In embryo daughter sporocysts about 0.1 mm in length the body cavity is well defined and the inner layer forms a large anterior plug. Such embryo daughters contain about 12 to 16 germinal cells and a few embryos. Daughters of *C. elephantis* at the time they escape from the mothers contain about 25 to 35 cercarial embryos and some germinal cells attached to the wall. The largest embryos at this stage are still very small but after the daughters have reached a size of from 1 to 2 mm they develop rapidly into cercariae, the first of which escape from the snail host about 5 to 6 weeks after infection. The pattern of germinal development in the germinal sacs of the spirorchiids is similar to that in the schistosomes. It is characterized by very rapid multiplication of the germinal cells in the early stages of development of both the mother and daughter sporocysts. No germinal masses are produced. The germinal cells become scattered along the wall of the body cavity and continue to divide and produce new embryos in the mother sporocysts long after the first daughters escape. In the daughter sporocysts new embryos are still being produced when the infections have become mature and the first cercariae have escaped.

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Further Studies on the Germinal Development in the Sporocysts of a Bird Schistosome, *Trichobilharzia stagnicolae* (Talbot 1936)*

W. W. CORT, D. J. AMEEL, AND ANNE VAN DER WOUDE

Cort and Olivier (1943) studied the germinal development in the mother and daughter sporocysts of *Trichobilharzia stagnicolae* (Talbot, 1936) in natural infections in *Stagnicola emarginata angulata* (Sowerby). Their observations were made in the early summers of 1940 and 1941 on immature infections in full grown adult snails. In some of these infections large immature mother sporocysts were present, but no early stages were found. They also studied the embryonic stages of the daughter sporocysts inside the mothers and their development after they had established themselves in the digestive gland of the snail.

Recent studies on the germinal development in the sporocysts of schistosomes (Cort, Ameel and Van der Woude, 1953 and 1954 a and b) have cast doubt on some of the observations that Cort and Olivier presented in their 1943 paper. Therefore, we decided to undertake further work on the development of the sporocysts of this species using experimental infections in juvenile snails. A preliminary account of his work has already been published in our general review on germinal development (Cort, Ameel, and Van der Woude, 1954 a).

MATERIAL AND METHODS

The adults of *T. stagnicolae* are only known from experimentally infected canaries (McMullen and Beaver, 1945). Therefore, during the summer of 1951 we exposed 14 canaries to the cercariae of this species. Two of the 14 canaries became infected and produced enough eggs to expose a number of laboratory raised juveniles of *S. emarginata engulata* to the miracidia. These canaries had been exposed repeatedly to large numbers of cercariae from several snail hosts from June 22 to June 30 and were first found to be positive on July 28 and August 7, respectively. The methods of study were the same as those used in all our recent researches on germinal development. We were careful not to overstain our preparations of immature sporocysts with neutral red since the use of such staining and the use of preparations which are too old makes it difficult to distinguish germinal from somatic cells and tends to break up small embryos (Cort, Ameel and Van der Woude, 1953).

DEVELOPMENT OF MOTHER SPOROCYSTS

In an earlier paper we described the arrangement of the germinal cells in the miracidium of *T. stagnicolae* (Ameel, Van der Woude, and Cort, 1953, fig. 3). They were found in a rather elongate compact group extending from just back of the large central nerve mass almost to the posterior end of the body. In eight specimens the number of germinal cells varied from 21 to 30 with an average of 22.

The mother sporocysts of *T. stagnicolae* in our experimental infections were found in the mantle of the snail. The larger ones were tightly attached to its tissues.

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In our sectioned material we located a mother sporocyst in a three day old infection. A longitudinal section through this mother which measured 0.12 by 0.03 mm is shown in figure 1. Its anterior end was attenuated and did not contain germinal cells. The body wall was clearly defined and its cells contained nuclei that were quite different in structure from those of the germinal cells. No embryos were present and the germinal cells which were irregular in shape were unusually large and mostly in the prophase, indicating that they were undergoing rapid division. Their cytoplasmic processes could be seen connecting them with the sporocyst wall and with each other. Since there were about 25 germinal cells in the longitudinal section shown in figure 1 and this sporocyst was round in cross section, it is evident that the germinal cells of the miracidium had multiplied considerably before any of them started to develop into embryos.

The four day old mother sporocyst shown in figure 6 measured 0.26 by 0.06 mm. About 60 germinal elements were counted in its body cavity, slightly more than half of which were small embryos.

Six mother sporocysts from 5 to 6 day old infections gave the following measurements: (1) 0.38 by 0.09 mm; 0.50 by 0.07 mm; 0.50 by 0.07 mm; 0.72 by 0.05 mm; 1.20 by 0.05 mm; and 1.40 by 0.10 mm. These measurements show a very great variation in size. In these mothers, especially the larger ones, there was a considerable increase in the number of embryos, and numerous germinal cells could be seen attached to the wall. Figure 2 is a longitudinal section through the anterior half of a 5 to 6 day old mother sporocyst. The sections of this mother showed daughter sporocyst embryos of different sizes and germinal cells in different phases of division held in a fibrous network. The inner layer of the body wall with its flattened nuclei was clearly defined. Figures 3a and b are consecutive cross sections through the posterior end of a mother sporocyst in a 5 to 6 day old infection. The sections of this mother also showed germinal cells and embryos of different sizes held in a fibrous network. In the small daughter sporocyst embryos in these sections the germinal cells could be distinguished from the somatic cells.

Mother sporocysts from 7 to 8 day old infections varied greatly in size. The smallest, which is shown in figure 7, measured 0.64 mm in length and 0.06 mm in width. Its wall was thick and its body cavity contained about 70 embryos, the largest of which had a diameter of about 0.03 mm. The germinal cells that are shown in the drawing at the anterior end and along the wall probably represent only a small fraction of those actually present. The embryos in this mother sporocyst were not tightly packed, and moved along the body cavity when it extended and contracted; but in most mothers examined at this stage the embryos were crowded in the body cavity. In a larger mother sporocyst from a 7 to 8 day old infection, which measured 1.70 by 0.05 mm, 110 embryos were counted in the body cavity. The largest of these had a diameter of about 0.04 mm and had not yet started to elongate. The largest of the 7 to 8 day old mother sporocysts that was studied had a length of about 3 mm and a width of about 0.07 to 0.08 mm in its widest portions. It contained 20 compact groups of daughter sporocyst embryos which were separated from each other by constricted places. The wall, even in a mother sporocyst this large, was quite thick and germinal cells and small embryos could be seen attached to it. The embryos in the groups varied greatly in size, were tightly attached to each other, and each group moved as a unit in the body cavity. The largest daughter sporocyst embryos in this mother were somewhat elongate with a size of about 0.07 by 0.05 mm, but had not yet assumed the characteristics of daughter sporocysts.

Fig. 1. Longitudinal section of a mother sporocyst, 0.12 by 0.03 mm from a three day old infection.

Fig. 2. Longitudinal section of the anterior part of a mother sporocyst from a 5 to 6 day old infection.

Fig. 3 a and b. Consecutive cross sections of the posterior end of a mother sporocyst from a 5 to 6 day old infection.

Fig. 4. Longitudinal section of a small daughter sporocyst.

Fig. 5. Longitudinal section of a young daughter sporocyst next to the wall of the mother.

In a series of mother sporocysts 8 to 10 days old there was a still greater variation in size and in the degree of development of the daughter sporocyst embryos. Only a few of the smaller mothers of this age that were about 1 to 2 mm long could be isolated unbroken. In them there were no active daughter sporocyst embryos that appeared ready to escape. The germinal material in some of these mothers was in a single mass extending the whole length of the body cavity. However, in most of the larger ones the embryos were distributed along the length of the body cavity in compact groups separated by constrictions. In each group there was usually at least one embryo that was beginning to elongate. This was usually in the center of the mass and had embryos of various sizes attached tightly to it and to each other. In some of the largest mother sporocysts in this series there were active daughter sporocysts moving freely in the body cavity which were 0.3 to 0.4 mm in length and appeared ready to escape. Their activity had broken up the larger masses of germinal material into smaller irregular groups of various sizes, some of which were composed only of embryos and others of embryos and varying numbers of germinal cells. These groups of germinal material floated freely in the body cavity mixed with the larger daughter sporocyst embryos. There were also germinal cells, very small embryos and, in some cases, even a few small compact groups of germinal material attached to the wall. These germinal elements were difficult to see in living specimens but showed up clearly in sections. The development of mother sporocysts in older experimental infections was not followed on account of lack of material.

DEVELOPMENT OF DAUGHTER SPOROCYSTS

The small daughter sporocyst embryos of *T. stagnicolae* in the germ ball stage (Figs. 2 and 3 a and b) are like those previously described in the mother sporocysts of *Schistosomatium douthitti* and *C. elephantis* (Cort, Ameel, and Van de Woude, 1953 and 1954 b). A daughter sporocyst embryo that had begun to elongate is shown in figure 8. It measured 0.086 by 0.045 mm and had 16 to 20 germinal elements in its body cavity, three of which were 2 to 4 cell embryos. In another daughter sporocyst embryo which was slightly larger, 0.12 by 0.04 mm, there were four cercarial embryos of slightly larger size (Fig. 9). At this stage the thin outer layer of the sporocyst wall with its flattened nuclei can be clearly seen, and the anterior plug formed of cells of the inner layer of the body wall, which is characteristic of young daughter sporocysts of schistosomes and spirorchiids, was conspicuous. Figures 4 and 5 are longitudinal sections of slightly larger daughter sporocyst embryos. Figure 4 shows clearly the difference in structure of the nuclei of the cells of the inner layer of the body wall and anterior plug, and those of the germinal cells. These sections also show the characteristic spination at the anterior end. The section shown in figure 5 is cut at such an angle that the anterior plug of body wall cells is not shown, but it is particularly interesting since it lies next to the wall of the mother sporocyst to which germinal cells and small embryos are attached.

A larger daughter sporocyst embryo, 0.19 by 0.05 mm, is shown in figure 10. It showed a considerable increase in the number of germinal elements since about 50 small embryos could be counted in its body cavity. In it, a number of germinal cells could also be made out and more were probably present.

A large series of free daughter sporocysts which varied in length from 0.25 to 0.50 mm were examined from infections from 15 to 17 days old. The body wall in these sporocysts was still quite thick and the anterior plug of body wall

Fig. 6. Four day old mother sporocysts, 0.26 by 0.06 mm.
Fig. 7. Mother sporocyst, 0.64 by 0.06 mm from a 7 to 8 day old infection.
Fig. 8. Daughter sporocyst embryo, 0.086 by 0.045 mm.
Fig. 9. Daughter sporocyst embryo, 0.12 by 0.04 mm.
Fig. 10. Daughter sporocyst embryo, 0.19 by 0.05 mm.
Fig. 11. Daughter sporocyst, 0.41 by 0.04 mm.
Fig. 12. Daughter sporocyst containing only a few large cercarial embryos.
Fig. 13. Daughter sporocyst, 0.52 by 0.05 mm.

cells though variable and, in a few cases almost completely absent, was in most of them still quite prominent. In some of the largest daughter sporocysts of this age cercarial embryos were elongate although they did not yet show the division into body and tail. In these daughters, because of the thickness of the body wall, it was difficult to see the germinal cells and small embryos attached to the inside of the wall in living material. The cercarial embryos in all these daughters were tightly packed in the body cavity and smaller embryos were wedged into spaces between larger ones. Figure 11 shows a daughter sporocyst 0.41 by 0.04 mm in which more than 50 cercarial embryos were counted in the body cavity, the largest of which was about 0.025 mm in diameter. The daughter sporocyst shown in figure 12 had about 35 embryos in its body cavity, the largest of which measured 0.050 by 0.025 mm. In some daughters, even fewer cercarial embryos were present, as for example, one about 0.26 mm long which had only about 20 embryos in its body cavity. Seven of them were large filling about nine-tenths of the body cavity with most of the small ones near the anterior end. In contrast to this, about 50 embryos were counted in another daughter that measured 0.30 by 0.04 mm. The sporocyst shown in figure 13 which measured 0.52 and 0.05 mm had at least 50 cercarial embryos in its body cavity, the largest of which was 0.075 by 0.035 mm, but did not show the division into body and tail. One of the largest sporocysts in these infections in which none of the cercarial embryos showed a division into body and tail was about 1 mm in length with 30 embryos in its body cavity. In all the free daughter sporocysts in the 15 to 17 day old infections the embryos were tightly packed together and at all levels small embryos were wedged between the larger ones. Examination of sections showed numbers of germinal cells and small embryos attached to the inside of the wall.

The daughter sporocysts were studied in several infections 19 to 20 days old. There was variation in the degree of development of the daughter sporocysts in these infections. However, most of them were large and contained well-developed cercarial embryos with eyespots. In a few cases, there were a few fully developed active cercaria that appeared about ready to escape. Some of the daughter sporocysts in these 19 to 20 day old infections were of about uniform thickness throughout their length and were crowded for their whole length with closely packed cercarial embryos of different sizes. In others, the cercarial embryos were in a series of compact groups which were separated from each other by constrictions. Germinal cells and very small embryos, or even small compact groups of embryos, could be seen on careful examination attached to the inside of the wall. In the few cases where active cercariae were present which moved around in the body cavity of the daughter sporocysts, the larger groups of embryos were broken up into smaller groups. The fact that a few fully developed active cercariae were present in 19 to 20 day old infections suggested that if these infected snails had been isolated before examination some cercariae would have escaped.

Large sporocysts containing active cercariae were studied from several infections that were about three weeks old. All contained some free floating groups of germinal material of differing sizes and number of elements. In some of these all the components were embryos, but in others varying numbers of germinal cells could be made out attached to the embryos. In these sporocysts some germinal cells, embryos and small groups of embryos could be seen attached to the inside of the wall. No older experimental infections were available for study.

Discussion

In the youngest mother sporocysts of *T. stagnicolae* found in experimental infections there appears to be a stage of multiplication of germinal cells before any of them develop into embryos. However, small embryos are present in some three day old mothers and they increase rapidly in number in slightly later stages. The growth of the mother sporocysts is rapid and sporocysts the same age vary greatly in size. The growth and multiplication of the germinal elements keeps pace with the increase in size of the mother sporocysts so that in most of them the body cavity is crowded with daughter sporocyst embryos in various stages of development which are usually attached tightly together. In mother sporocysts in 8 to 10 day old infections the germinal material forms either a single mass filling the whole body cavity or a series of smaller masses separated from each other by constrictions of the body wall. These masses of germinal material are composed of daughter sporocyst embryos of all sizes mixed together, as well as a few germinal cells either wedged between the embryos or attached to them. At this stage, germinal cells, small embryos, and even small groups of germinal elements are present attached closely to the inside of the wall. In mothers in which there are actively moving daughters the large masses which fill the body cavity are broken up into individual embryos and smaller groups of germinal elements of various sizes and composition that float freely in the body cavity. These small irregular groups of germinal material are not true germinal masses like those in the mother sporocysts of the strigeoids (Cort and Olivier, 1941), but are merely embryos and germinal cells that remain temporarily attached to each other after the breaking up of the larger masses by the activity of the daughter sporocysts.

The structure of the small daughter sporocyst embryos in the "germ ball stage" in the mother sporocysts of *T. stagnicolae* is like that of the same stages in other schistosomes and spirorchiids. When the daughter sporocysts begin to elongate they are also very similar to those of these species. They have a well defined body cavity in which a few of the germinal cells have already developed into embryos. The cells of the inner layer of the body wall form a plug at the anterior end. Daughter sporocyst embryos, 0.2 to 0.3 mm long, become active in the body cavity of the mothers and are ready to escape. In their body cavities are crowded cercarial embryos of various sizes and germinal cells, which are wedged between the embryos, attached to them, or attached to the wall. In daughter sporocysts outside the mother in the digestive gland of the snail the germinal material still fills the body cavity. As the daughters grow larger the mass of embryos in the body cavity may be divided as in the older mother sporocysts into groups by constrictions of the wall. As the largest cercariae become active they break up the masses of germinal material into small irregular groups composed either only of embryos or of embryos and varying numbers of germinal cells attached closely together. These small groups of adhering germinal material cannot be interpreted as germinal masses, but like those found in older mother sporocysts are merely groups of germinal elements temporarily attached to each other. Also, in the larger daughter sporocysts, germinal cells, small embryos and small groups of germinal material are found attached to the inside of the wall. The finding of active daughter sporocysts in mothers of *T. stagnicolae* only 8 to 10 days old, indicates that they will begin to migrate into the digestive gland of the snail host at about this time. Development of the daughter sporocysts in the digestive gland is very rapid, since the first cercariae become fully developed and active

in 19 to 21 days. This development is more rapid than that of the spirorchiid, *C. elephantis*, or of *S. douthitti*.

The development of the mother sporocysts of *T. stagnicolae* in the experimental infections is very much like that of the spirorchiid, *C. elephantis*. However, in these species the mother sporocysts are quite different from those of *S. douthitti* in which the daughter sporocysts develop synchronously and fill only a small part of the thin-walled inflated mother sporocysts. The daughter sporocyst embryos of *T. stagnicolae* are very similar to those of *C. elephantis* and *S. douthitti* (Cort, Ameel and Van der Woude, 1953; 1954 a, b). However, they differ from the descriptions given by Cort and Olivier (1943) of the same stages in natural infections of *T. stagnicolae*. It was suggested in an earlier publication (Cort, Ameel, and Van de Woude, 1953) that as in the earlier studies on *S. douthitti* (Cort, Ameel, and Olivier, 1944) this discrepancy in the descriptions in the earlier studies was probably due to staining too heavily with neutral red and the examination of preparations which were too old. In such preparations of embryo daughter sporocysts, the germinal cells cannot be distinguished from the cells of the inner layer of the body wall and the anterior plug, and small embryos which are frequently broken up cannot be distinguished from germinal cells.

The descriptions of Cort and Olivier (1943) of the mother sporocyst and larger daughter sporocysts of *T. stagnicolae* also differed from our observations in the experimentally infected juveniles. The youngest mothers that they found were thin walled, sausage-shaped sacs about 3 mm in length. They were usually located in the tissues on the surface of the organs of the snail in front of the digestive gland, particularly on the kidney and stomach. The germinal material which filled only a small portion of the body cavity was suspended in the lumen by strands of tissue and consisted of single germinal cells, groups of germinal cells, and irregular masses consisting of small embryos and germinal cells. In older mothers the germinal material consisted of irregular groups of embryos and germinal cells and larger single embroys some of which were active and moved freely in the body cavity. Even in the largest mothers from which daughters were already escaping the embryos filled only a part of the body cavity. In daughters that had started their growth in the digestive gland of the snail the germinal material was attached by protoplasmic processes to the wall and filled only a part of the body cavity. Numerous irregular groups of germinal material were present consisting of small embryos or small embryos and germinal cells. Older daughters were filled with large numbers of active cercariae and large cercarial embryos which nearly filled the body cavity and had only a few groups of germinal material. The groups of germinal material in both mother and daughter sporocysts were considered by Cort and Olivier to be floating germinal masses.

One of the most striking differences between the two descriptions is the location of the mother sporocysts in the snail host. In the small juvenile snails they were located in the mantle and as they develop become tightly imbedded in its tissues. In the large mature snails they were usually found attached to the tissues of the organs in front of the digestive gland, particularly the kidney and stomach. It might be suggested that in the older snails the tissues of the mantle are too tough for the entrance of the miracidium and the development of the sporocyst. This difference in location may also be a factor in the differences in structure of the mothers in the large and small snails. At least, it seems probable that the differences are due to development in large mature snails in the natural infections studied by Cort and Olivier (1943). In the large snails much more food is available and there is more room for the

development of both the mother and daughter sporocysts. Under these more favorable conditions the increase in size of the sporocysts is very rapid and germinal development lags far behind. The rapid growth of the wall of the mother and daughter sporocysts would tend to pull the germinal material which is held in a network of fibers out into the lumen so that it appears to be almost in the center of the body cavity attached to long strands of the fibrous network. The multiplication of the individual germinal cells to form groups and the development of some of them into embryos produces "pseudo-germinal masses" in both mother and daughter sporocysts which are really only temporary groups of attached germinal elements and entirely different from the true germinal masses of the strigeoids. With the activity of the fully developed daughter sporocysts in the mothers and the cercariae in the daughters these "pseudo-germinal masses" are freed from the fibrous network and float freely in the body cavity of the sporocysts. Evidence for this explanation is that the mother and daughter sporocysts in the large mature snails grow to a much larger size than those that develop in experimental infections in small juveniles, and their walls are much thinner. Even the daughter sporocysts that are ready to escape from the mothers are considerably larger in the natural infections in the large snails.

In the experimental infections of *T. stagnicolae* in the small juveniles the development of the germinal material keeps pace with the expansion of the lumen of the body cavity of the sporocysts, so that it appears as a large mass extending for the whole length or in larger sporocysts as a series of masses separated by constrictions of the wall. When these masses are broken up by the growth and activity of the daughter sporocysts and fully developed cercariae small groups of germinal material are freed which correspond to the "germ masses" Cort and Olivier (1943) described in the infections in the large snails. There is, therefore, not the slightest evidence that true germinal masses like those of the strigeoids play a part in germinal development of *T. stagnicolae.* Individual germinal cells develop directly into embryos, and germinal cells persist mixed with embryos and attached to the wall to provide for the production of new embryos throughout the reproductive life of the mother and daughter sporocysts. The germinal development in the sporocysts of *T. stagnicolae* is like that of the schistosome, *S. douthitti,* and the spirorchiid, *C. elephantis,* and differs fundamentally from that of the strigeoids in which true persistent germinal masses play the most important part in the multiplication of the germinal cells (Cort and Olivier, 1941).

SUMMARY

A study was made of the germinal development of a bird schistosome, *Trichobilharzia stagnicolae* in small laboratory raised juveniles of *Stagnicola emarginata angulata.* The miracidia for infecting the snails were obtained from experimentally infected canaries.

In the miracidium-mother sporocyst stage there is a rapid multiplication of germinal cells some of which very early begin to develop into embryos. The embryos develop slowly in the early stages and most of the division of germinal cells is completed before they begin to take on the shape of daughter sporocysts. In the embryos of daughter sporocysts the multiplication of the germinal cells is extended over a longer period. The development in the snail host is very rapid. Daughter sporocysts begin to escape from the mother in about 10 days and the first cercariae are mature at about 21 days. Multiplication of germinal cells takes place in all parts of the body cavity in both mother and daughter sporocysts, and no germinal masses are developed. However, groups

of germinal elements temporarily attached to each other are found at various stages in mother and daughter sporocysts both free and attached to the wall.

It was suggested that the size of the snail host has considerable effect on the development of both mother and daughter sporocysts. In natural infections in full grown snails the sporocysts grow to larger size and develop into thin walled inflated sacs. In experimental infections in very small snails, the germinal material tends to be very crowded in the sporocysts.

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Cestodes of Sharks and Rays in Southern California

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Little work has been done on cestodes of fishes from the western coast of North America. Sleggs (1927) recorded four species from fishes of the California coast and Pintner (1930) recorded two tetrarhynchs from the same region, while Hart (1936 a, b), Hart and Guberlet (1936), Kay (1942), and Wardle (1932, 1935) reported on several species from the Puget Sound Region.

Most of the fishes which furnished the material for this study were taken in 1950, '51, and '52 in Mission and San Diego Bays, inlets of the sea near San Diego, California. A few other records are included from points as far north as Monterey.

Types of all new species have been deposited in the Zoological Laboratory of the U. S. Bureau of Animal Industry in Beltsville, Maryland.

The studies were made in the laboratories of the San Diego Zoological Society and the University of Maryland to whom my thanks are due for many courtesies rendered.

TECHNIQUE

The sharks and rays examined were mainly taken on set lines, although a few were seined. The small teleosts in which larval stages may occur were also seined.

Worms were fixed in Dubosq-Brazil's fluid (alcoholic Bouin's) or 10% formalin. Ehrlich's hematoxylin was usually used for staining but a few preparations were stained with aceto-carmine and Semichon's carmine (Morgan and Hawkins 1949, p. 355). Both whole mounts and sections were employed. All measurements given are in millimeters and were made on preserved specimens.

ORDER TETRAPHYLLIDEA

Family Phyllobothriidae

Echeneibothrium flexile (Linton, 1890)

SYNONYM: *Rhinebothrium flexile* Linton, 1890

HOSTS: *Urobatis halleri* (Cooper), round sting ray
Holorhinus californicus (Gill), California sting ray.

DISCUSSION: Linton (1890, p. 769) gives the number of loculi tentatively as 60 to 80. An exact count of the loculi is difficult. The range in my specimens is apparently from 30 to 42, with 38 as the most frequent number. There is occasionally a difference between numbers of the same pair. Both Linton and Baer (1948) describe the cirrus as echinate, a character not verifiable in my material which was not wholly satisfactory to reveal such condition.

Echeneibothrium tumidulum (Rud., 1819)

HOST: *Urobatis halleri* (Cooper)

DISCUSSION: Wardle and MacLeod (1952, p. 240) note that this specific name at present actually includes a group of species and "is applicable to all the non-rostellate forms in which each bothridium has a double series of loculi." The name is used here in this sense. There are apparently two species in my collections.

Echeneibothrium minimum van Beneden, 1850

HOST: *Urobatis halleri* (Cooper)

DISCUSSION: A single, non-gravid specimen was collected. It is only 6 mm. long but has 23 proglottids. It differs from van Beneden's material in having 13 to 14 loculi rather than 8 to 10. A myzorhynchus is apparently absent but van Beneden states it is "peu prononcé dans cette espèce." There are 10 to 11 testes as compared with 20, the approximate number reported by Joyeux and Baer (1936) for this species.

Echeneibothrium multorchidum n.sp. (Figs. 1 and 2)

HOST: *Urobatis halleri* (Cooper)

SPECIFIC DIAGNOSIS: With the characters of the genus. Bothridia stalked, with longitudinal and transverse partitions dividing each into approximately 38 loculi. Average size of two bothridia in one specimen 0.58 long by 0.23 wide. Neck absent or short. First segments wider than long, last segments longer than wide, one of the latter measuring 1.39 by 0.27. Gonopore in the anterior third of proglottid. Testes 30 to 45; 0.025 in diameter (average of ten), flattened anterior-posteriorly.

DISCUSSION: The number of testes, in combination with its other characters distinguishes this species from any heretofore described.

TYPE: U. S. Nat. Mus. Helm. Coll. No. 49098.

Anthobothrium laciniatum Linton, 1890

HOST: *Urobatis halleri* (Cooper)

DISCUSSION: My specimens resemble those of Linton except that the bothridia are sessile and there are no laciniae. Several specimens of Linton's material which I have examined in the collection of the U. S. National Museum show distinct and constant laciniae but Linton (1924) reports them as incon-

stant structures. The bothridial stalks are not very satisfactory structures for differentiating species since when contracted they may appear as absent.

Anthobothrium oligorchidum n.sp. (Figs. 3-4)

HOST: *Urobatis halleri* (Cooper)

SPECIFIC DIAGNOSIS: With the characters of the genus. Bothridia wider than long; 0.35 by 0.24; with regular or slightly scalloped edges; stalked. Neck varying from 1.02 to 3.36 (average of five specimens, 2.15). Segments smooth. Gonopore slightly posterior to the middle of the segment. Cirrus sac extending to the middle of the segment. Testes 12, in the anterior 2/3rds of the segment, 0.031 by 0.032 (average of ten).

DISCUSSION: Although the few specimens collected, all from *Urobatus halleri*, are inadequate for a full description, the small number of testes distinguishes this species from others described in the genus.

TYPE: U. S. Nat. Mus. Helm. Coll. No. 49096.

Family Onchobothriidae

Acanthobothrium dujardini Van Beneden, 1849

HOSTS: *Urobatis halleri* (Cooper)

Rhinobatos productus (Ayres), guitarfish.

DISCUSSION: The size of the cirrus sac is a striking feature of this species. Three posterior segments averaged 0.169 in width while the corresponding cirrus sacs averaged 0.096 to 0.106.

Verma (1928) described *A. semnovesiculum* from *Trygon septen* in India. It resembles *A. dujardini* except in its voluminous seminal vesicle, shorter hook length, and smooth neck. Southwell (1930) considers it a synonym of *A. dujardini* while Baer (1948) considers it to be *A. benedeni*, and Wardle and MacLeod (1952) recognize it as a valid species. The seminal vesicle is merely the terminal portion of the vas deferens and when expanded with sperm assumes the form described and figured by Verma. Absence of neck spines might result from their loss in preparing the material.

Acanthobothrium crassicolle Wedl, 1855

HOST: *Urobatis halleri* (Cooper)

DISCUSSION: My specimens are identified as *A. crassicolle* with some uncertainty partly because various descriptions of *crassicolle* do not agree. Therefore a few observations on my material should be made. The number of segments is over 100. The neck is long and smooth; in two specimens with an average length of 12 mm., the neck averaged 8.75. Bothridia are 0.50 to 0.80 long by 0.30 to 0.40 wide; accessory suckers apparently absent. Average measurements on hooks: length 0.145; handle 0.067; inner prong 0.085; outer prong 0.086 (measurements made along the chord, not the arc of the hook). The inner prong of the hook bears a conspicuous tubercle and the bothridia have an extended attachment, as in *A. crassicolle*. Gonopore slightly posterior to middle of the segment. Testes 50 to 60. Cirrus spined.

The material differs from *A. crassicolle* in its smooth neck and apparent absence of any suckers on the bothridia.

Acanthobothrium parviuncinatum n.sp. (Figs. 5-7)

HOSTS: *Urobatis halleri* (Cooper)

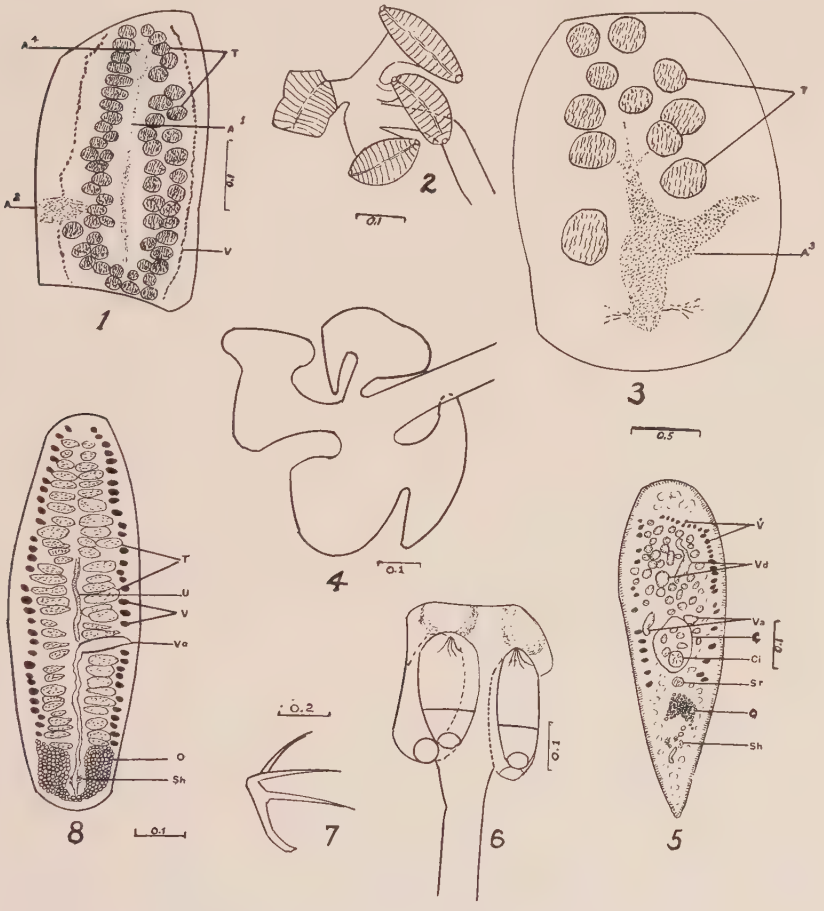
Gymnura marmorata (Cooper), butterfly ray.

Larvae in a crab, *Hemigrapsus* sp.

SPECIFIC DIAGNOSIS: With the characters of the genus. Bothridia 0.29 by 0.16 (average of five); apical sucker present. Average measurements of hooks: length 0.087; handle 0.017; inner prong 0.07; outer prong 0.062 (meas-

urements made along the chord, not the arc of the hook). Neck smooth, its length depending on the state of contraction of the specimen.

Proglottids many. Average size of five end segments (mature but not gravid) 0.64 by 0.19. Gonopore anterior to middle of the segment. Cirrus sac large, extending to middle of segment. Average of five, 0.09 by 0.07. Cirrus heavily spined, spines measuring 0.007 to 0.010. Testes 12 to 14, in anterior



1. *Echeneibothrium multorchidum.* Immature proglottid.
2. *E. multorchidum.* Scolex.
3. *Anthobothrium oligorchidum.* Immature proglottid.
4. *A. oligorchidum.* Scolex.
5. *Acanthobothrium parviuncinatum.* Nearly mature proglottid.
6. *A. parviuncinatum.* Scolex.
7. *A. parviuncinatum.* Hooks.
8. Partly mature proglottid frontal section of *Parachristianella trigonis.*

All drawings were outlined with the aid of a camera lucida; the details added free hand.

A^1—anlage of vas deferens and vagina; A^2—anlage of cirrus sac and vagina; A^3—anlage of genital complex; A^4—anlage of ovary; C—cirrus sac; Ci—cirrus; O—ovary; Sh—shell gland; T—testes; V—vitellaria; Va—vagina; Vd—vas deferens.

half of segment. Ovary large; in one proglottid measuring 0.32 by 0.256 with wings 0.160 and 0.112 in length and the isthmus 0.028 in thickness.

Discussion: This species is characterized by its small hook, its few testes, and numerous segments.

Type: U. S. Nat. Mus. Helm. Coll. No. 49095.

Onchobothrium pinguicollum (Sleggs, 1927)

Host: Indeterminate.

This species was described by Sleggs from a "skate" at Monterey.

Phyllobothrium radioductum Kay, 1942

Host: *Triakis semifasciata* Girard, leopard shark

Order Tetrarhyncha

Family Eutetrarhynchidae

Christianella trigonis-bucconis (Wagener, 1854)

Hosts: Adults in *Urobatis halleri* (Cooper)

Larvae in *Callianassa,* a shrimp and in *Hemigrapsus* or *Pachygrapsus,* crabs

Discussion: These cestodes agree well with the description by Joyeux and Baer (1936, p. 117-119) except that the hooks are arranged in alternating half circles of eight as Dollfus (1942, p. 220) has stated. Also the bothridia are not notched posteriorly; but Wagener's original figure did not show such notching nor does Joyeux and Baer's figure 69. Measurements of my specimens are slightly larger than those given by Joyeux and Baer. The number of segments varies from six to nine, and the number of testes averages about fifty. The retractor muscle is inserted at the base of the bulb.

Discovery of the larvae of this species in shrimps and crabs is of some interest. Present records of tetrarhynchs in Crustacea are meagre. Dollfus (1942, p. 72) lists 18 species of decapod Crustacea harboring various species of tetrarhynchs. Of these only three are definitely identified to species and only one is known from the Pacific, in the Inland Sea of Japan (Yamaguti, 1934).

Parachristianella trigonis Dollfus, 1946 (Fig. 8)

Host: *Urobatis halleri* (Cooper)

Discussion: Measurements of my specimens agree well with those of Dollfus. The large hooks of the proboscids are somewhat smaller than in his material, measuring 0.014 in both height and basal length. The points curve backward and the posterior root is a trifle longer than the anterior. The cells in the bulbar cavity are very evident. They are arranged in much the same way as Dollfus describes them in the larva of *P. trigonis* but are limited to the bulbs and apparently do not occur in the sheaths as he reports. The "organe prebulbaire" was not found. The retractor muscle is inserted at the base of the bulb. Dollfus did not have mature segments so the following observations are given.

The number of segments varies from six to ten. There is a bilobed, bilaminate ovary occupying the posterior fifth of the segment with the wings extending forward on either side for about 3/5ths the length of the gland. The vitellaria, which lie inside the longitudinal muscles, surround the proglottid in an irregularly interrupted sheath. The smooth cirrus and the vagina unite to open through a common gonopore in the posterior third of the proglottid. There are more than 100 testes arranged in two rows extending from the anterior end of the proglottid to the level of the ovary. They are flattened anterio-posteriorly; the frontal section (Fig. 8) shows 60 testes in one plane.

Lacistorhynchus tenuis (van Beneden, 1858)

HOST: *Triakis semifasciata* Girard, leopard shark
Plerocercoids in *Cymatogaster,* surf perch

A separate note is being published regarding the experimental infection of the leopard shark with this cestode.

Nybelinia (*Syngenes*) *palliata* (Linton, 1924)

SYNONYM: *Tetrarhynchus bisulcatus*

HOST: *Lamna ditropis* Hubbs and Follett, 1947, a shark

This species was reported by Sleggs (1927). It is known from Woods Hole and has been reported from the Asiatic Pacific by Pintner (see Dollfus, 1942, p. 193).

INCOMPLETELY IDENTIFIED FORMS

A tetrarhynch larva is reported by Pintner (1930) from the skin of *Chimaera collei* Lay and Bennett, ratfish, at Pacific Grove, California.

An immature, tenioid tapeworm was found in the spiral value of *Urobatis halleri.* The 24 hooks in a single circle are suggestive of *Choanotacnia* or *Paricterotaenia.*

Christianella sp.

HOST: probably *Urobatis halleri* (Cooper)

DISCUSSION: In my collection was a single specimen of a tetrarhynch for which data were incomplete so I cannot be certain of the host. It was probably *Urobatis halleri.* Condition of the specimen is inadequate for a complete description. It is apparently an undescribed species of *Christianella.* Total length approximately 1.5. Number of segments three, the last segment, partly ripe, measuring 0.54 by 0.12. Scolex 0.74 by 0.14. Bothridia 0.13. Pars bothridialis 0.15 by 0.2; pars vaginalis 0.18; pars bulbosa 0.40; pars post bulbosa 0.14 wide; diameter of bulbs 0.06. Hooks all similar, about 20 to 25 in full circle; length 0.003 to 0.004. Testes numerous.

The most characteristic feature of the worm is the presence of heavy spines (maximum length 0.0084) which cover the scolex from the bothridia back to the posterior end of the scolex where they gradually disappear.

SUMMARY

Eighteen cestodes are listed from Selachians of Southern California. Most of these represent new host and locality records, and three are new species.

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A Note on the Life Cycle of *Lacistorhynchus tenuis* (Van Beneden, 1858), A Cestode of the Leopard Shark

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Plerocercoids of a tetrarhynchid cestode, considered to be *Lacistorhynchus tenuis* (Van Beneden, 1858), occur commonly in the surf perch, *Cymatogaster aggregata* Gibbons, in San Diego Bay, California. These were transferred to the leopard shark, *Triakis semifasciata* Girard, as shown in the following experiment.

Several young sharks were obtained from a gravid female speared by a fisherman about 30 miles from the Scripps Institution at LaJolla, California, in May, 1949. Five of these unborn young were kindly given to me by Dr. Carl L. Hubbs of the Institution. Three of these were used in a feeding experiment, the other two being kept as controls.

The three experimental sharks were fed tetrarhynch larvae from *Cymatogaster* from about May 30 to July 9. One of these sharks was killed on June 4, the other two on July 16. The former contained one immature tetrarhynch. One of the two latter contained a few, the other many tetrarhynchs. The controls were both negative. The cestode was considered to be *Lacistorhynchus tenuis*.

The largvae show that the post-bulbar swelling on the scolex is an inconstant character as concluded by Dollfus (1942, pp. 325, 330).

Lacistorhynchus tenuis is known as an adult from the following hosts of the North Atlantic and the Mediterranean (Dollfus, 1942, p. 344): *Galeus canis, Mustelus hinnulus, Mustelus canis, Acanthias acanthias Vulpecula marina, Spinax niger, Raja batis*. It has not apparently been reported previously from *Triakis*, nor from the Pacific. Its plerocercoids have been reported from more than 60 species of teleosts.

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Lyophilization and Low Temperature Studies with the Bulb and Stem Nematode *Ditylenchus dipsaci* (Kühn 1858) Filipjev[1]

J. E. BOSHER[2] AND W. E. MCKEEN[3]

The survival of plant-parasitic nematodes in the field under extremely low temperature conditions has been frequently observed, but the percentage survival and the conditions governing such survival are little understood. For example, Bessey (1911) records continuous infection of ginseng by the root-knot nematode in Michigan where the soil had frozen to depths of three feet, and the survival of this nematode in peony roots exposed to −30° F., although Tyler (1944) states that all stages of this organism are killed by exposure to −4° F. for a two-hour period. Ramsbottom (1918) records the survival of the bulb and stem nematode in narcissus bulbs exposed to 37° F. of frost. Canadian and U.S.A. Plant Disease Survey records contain numerous references to the occurrence of nematodes on plants from regions where winter temperatures are normally extremely low, indicating that the occurrence of plant-parasitic nematodes is probably dependent upon the presence of host plants and not prevailing temperatures.

The bulb and stem nematode, *Ditylenchus dipsaci* (Kühn 1858) Filipjev, provides a convenient organism for laboratory studies in the form of narcissus "wool," consisting of clusters of the pre-adult stage of this nematode in large numbers, found in a dried state in the basal region of narcissus bulbs in dry storage. Hastings (1942) has determined the effective longevity of nematodes in this state under laboratory conditions to be not more than two years, but more recent observations indicate the possible extension of this period by modification of storage conditions.

The lyophilization technique, as used for the preservation of other micro-organisms, provides a convenient method for study of the effect on nematodes of extremely low-temperature freezing, vacuum dehydration, and storage *in vacuo*. Studies of longevity following treatment by this process have not been concluded, but data are presented of the effect on survival of the nematodes following freeze-drying while in the dry state and in the active state in various media, and of freezing without subsequent dehydration.

METHODS AND MATERIALS

The test material was placed in test tubes made of ¼" pyrex-glass tubing and frozen for a standard period of 20 minutes in a vacuum jar containing ethylene glycol, dry ice, and water at a temperature of −80° C. (−112° F.). Dehydration was by a duo-seal vacuum pump which registered a vacuum of .025 micron (.000025 mm. Hg.) for six hours. Vacuum was maintained while sealing the test tubes with a propane-oxygen flame. The material which was not dehydrated was allowed to thaw overnight in the laboratory before being decanted into 10 cc. of tap water in 50-mm. syracuse dishes. In most cases, it was necessary to rinse the material from the tubes by syringing with a capillary tube. Examination and counts of the revived nematodes were made at intervals from 2 to 48 hours after decanting. In some cases, observations were continued for ten days after treatment, but no revivals occurred after 24 hours.

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OBSERVATIONS AND RESULTS

The first series of tests included dry narcissus wool, dry wool moistened with beef serum or tap water immediately before treatment, active nematodes in water, and active nematodes placed in beef serum all of which were frozen to −80° C., vacuum dehydrated, and sealed in tubes *in vacuo*. One tube of each type was opened 20 hours after sealing, and a similar series was opened after storage for 28 days at room temperature. The results are presented in Table 1. Best survival was obtained when the dry wool was used. Nematodes that failed to survive contained huge vacuoles or air pockets which extended almost the full length of their bodies (Fig. 4).

TABLE 1.—The survival in various media and in various stages of activity of pre-adult nematodes (*Ditylenchus dipsaci*) which were frozen at −80° C., vacuum dehydrated and stored *in vacuo*.

	Stored *in vacuo*					
	20 hours			28 days		
Nematode state	2 hrs.*	6 hrs.*	24 hrs.*	2 hrs.*	6 hrs.*	24 hrs.*
Dry wool	20**	80**	80**	18**	80**	80**
Dry wool + beef serum	0	50	80	0	30	80
Dry wool + water	0	0	trace	5	30	30
Active in water	0	0	0	0	0	0
Active in beef serum	0	0	0	0	0	0

* Number of hours nematodes were in water after vacuum was broken.
** Percentage of nematodes that became active.

Beadlike vacuoles are commonly seen in the intestinal region of nematodes revived in water from the dried state (Figs. 2 and 3), but they do not normally involve the oesophageal region nor are they so extensive and continuous as in the case of the frozen specimns.

Since the nematodes moistened with beef serum were less injured than those maintained in water, it was considered that the large vacuoles may have resulted from freezing of water within the body cavity. Probably more water entered the nematode when it was bathed in water than when it was placed in beef serum because of the higher osmotic value of the serum. However, beef serum proved to be an unsatisfactory medium for nematodes since maximum revival occurred only after the original suspension was highly diluted, and contamination of the medium by other microorganisms readily occurred. The results of a second test which included a series moistened with a 50% sucrose solution are shown in Table II. A high percentage of the nematodes which were moistened with various solutions just prior to freezing survived, but no nematodes survived when frozen while in the active state. The sucrose solution proved to be more suitable than beef serum, because of its freedom from con-

TABLE 2.—The survival in various media and states of activity of nematodes subsequent to lyophilization

	Stored *in vacuo*					
	20 hours			12 days		
Nematode state	2 hrs.*	6 hrs.*	24 hrs.*	2 hrs.*	6 hrs.*	24 hrs.*
Dry wool	70**	80**	80**	80**	80**	80**
Dry wool + beef serum	5	80	80	0	70	70
Dry wool + 50% sucrose solution	70	80	80	30	90	90
Dry wool + water	3	4	4	0	30	30
Active in water	0	0	0	0	0	0

* Number of hours nematodes were in water after vacuum was broken.
** Percentage of nematodes that became active.

tamination, more rapid revival of nematodes in it, and the fact that it did not require high dilution. It was also found that vacuoles seldom occurred in nematodes frozen while in sucrose (Fig. 1).

In order to test the theory that the death of the nematodes was caused by

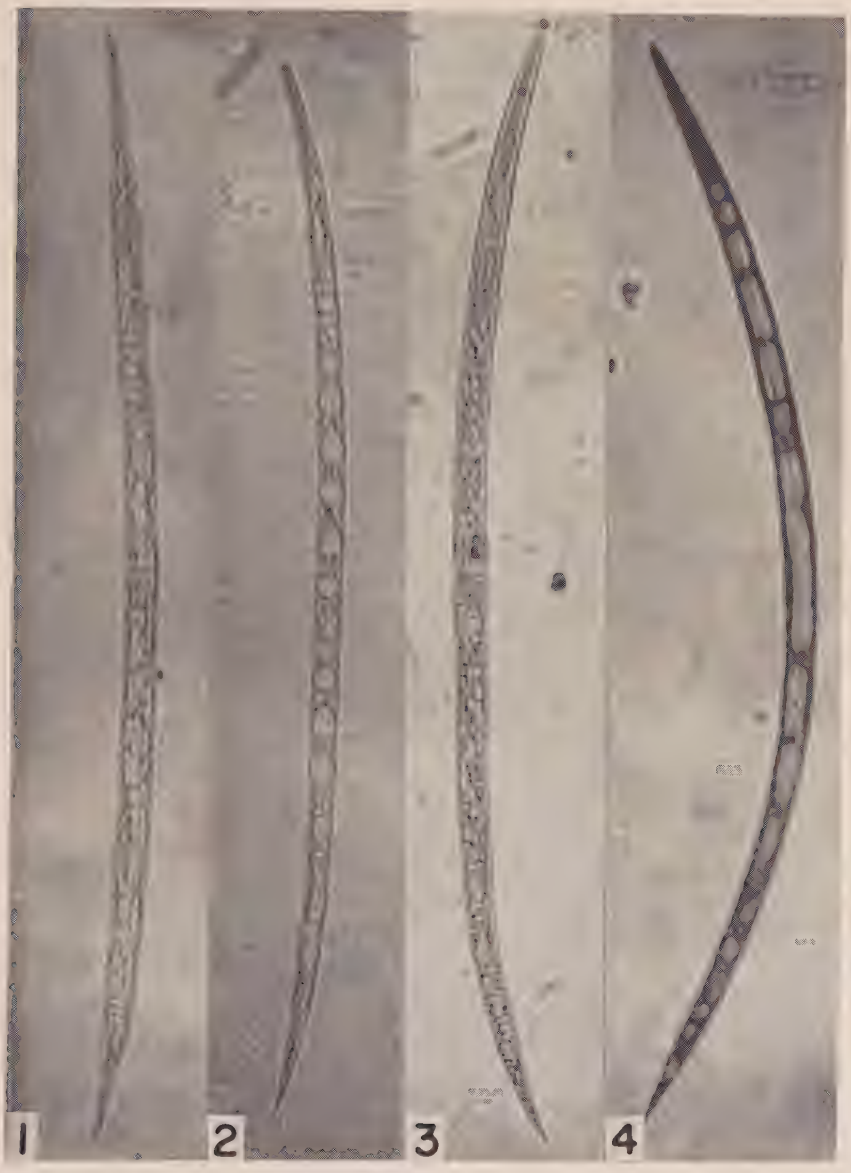

PLATE I.

Fig. 1. A nematode that survived freezing to —80° C. for 20 minutes while in 66% sucrose . Note absence of vacuoles. Fig. 2. A nematode that was revived from dry wool in water. Note beadlike vacuoles that do not extend into the oesophageal region. Fig. 3. A nematode that was revived from dry wool in water. No vacuoles present. Fig. 4. A nematode frozen to —80° C. for 20 minutes after it was revived in water. Observe that large vacuoles extend into the oesophageal region.

freezing of free water within the body tissues, nematodes were frozen at —80° C., without subsequent dehydration, in solutions containing various concentrations of sucrose and after they had been placed in water for various periods of time.

Table 3 shows that there was a positive relationship between the concentration of sucrose and the survival of nematodes after freezing at —80° C. and a negative relationship between survival and dispersion of nematodes. It will also be noted that the percentage survival dropped very rapidly as the length of time the nematodes were in water increased.

TABLE 3.—The effect of various sucrose concentrations and length of time in water on nematode wool, and the percentage survival, following freezing to —80° C. for 20 minutes, after 30 minutes in various concentrations of sucrose, and after various periods of time in water

	Before freezing		After freezing	
Nematode state	Condition of wool after ½ hour	Percentage revival after 4 hrs.	Stored 20 hrs.	Stored 10 days
Wool in 5% sucrose	dispersed	90	trace	trace*
Wool in 10% sucrose	dispersed	90	2**	trace*
Wool in 25% sucrose	partly dispersed	50	3	trace*
Wool in 50% sucrose	not dispersed	0	90	15* **
Wool in 66% sucrose	not dispersed	0	90	90
Wool in water 2 hours	dispersed		trace	0
Wool in water 1 hour	dispersed		trace	0
Wool in water ½ hour	dispersed		0	0
Wool in water 10 minutes	dispersed			trace
Wool in water 2 minutes	after 4 hours		70	40
Wool only			98	80

* Slight fungus growth on surface of media.
** Percentage revival.

DISCUSSION

When nematodes were in active condition in water or dilute solutions, they were unable to withstand very low temperature, and thus the lyophilization process could not be successfully carried out. It is probable that the occasional specimens surviving treatment while in water or weak solutions were within fragments of dry bulb tissue. The slower dispersal and motility of the nematodes in 25% sucrose solution and failure to disperse or become active in higher concentrations indicate that the absorption of water from the medium is reduced as the sugar concentration is increased, as might be expected. The fact that the percentage survival dropped as the period of time nematodes were in water lengthened, and as the concentration of sucrose was lowered, proves that the freezing temperatures injured their bodies after they had absorbed a certain amount of water.

When dry wool was placed in water, beadlike vacuoles sometimes appeared and remained indefinitely. However, in sucrose solutions above a certain concentration, vacuoles usually were absent, and freezing in this condition was not harmful to the nematode. Apparently, all the water is bound within the body, and no free pockets of water exist. Sucrose probably enters the body and may also aid in protecting nematodes against injury from low temperatures. It is very probable that organic matter surrounding nematodes may help protect them from injury during cold winter periods.

Since nematodes, under certain conditions, may survive the lyophilization treatment and be stored at room temperatures for periods of time, this technique appears to be of value for maintaining stock cultures and for enabling nematologists to have active nematodes for study at any time.

SUMMARY

Pre-adult larvae of the bulb and stem nematode, *Ditylenchus dipsaci* (Kühn 1858) Filipjev, have retained viability following lyophilization.

A high percentage of the nematodes, when in the dry state as narcissus "wool," or when moistened with beef serum or a concentrated sucrose solution, survived freezing for 20 minutes at a temperature of −80° C., followed by vacuum dehydration and storage *in vacuo* for a 28-day period.

In water, the nematodes were injured and killed by freezing to −80° C., with the formation of large vacuoles within their bodies, but this did not occur when the nematodes were in solutions with higher osmotic values, because less water entered the nematodes.

Lyophilization would be a useful technique for the study of some phases of nematode biology, and may be used for the preservation of certain types of nematodes in the living state.

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Bialovarium nocomis Fischthal, 1953 (Cestoda: Caryophyllaeidae) from the Hornyhead Chub, *Nocomis biguttatus* (Kirtland)*

JACOB H. FISCHTHAL

In a fish parasite survey of northwest Wisconsin Fischthal (1947) listed an unsegmented tapeworm, found in the small intestine of the hornyhead chub, *Nocomis biguttatus* (family Cyprinidae), as belonging to the "Cestodaria." Fischthal (1953b) indicated this identification as being incorrect and placed the worm in the family "Caryophyllaeidae." Subsequent comparisons with known genera of the family showed it to represent a new genus and species. In an abstract of a paper read at the 28th annual meeting of the American Society of Parasitologists, Fischthal (1953a) presented a preliminary description of this new tapeworm, and proposed the name *Bialovarium nocomis* for it.

One of 17 *Nocomis biguttatus* taken from Meadow creek, Barron County, on February 4, 1944, harbored a single mature tapeworm. Its uterus was devoid of eggs, but sperm were present in the vas deferens and vagina. The description presented below is based on this single specimen which was studied first as a whole mount, and then in serial cross sections. The worm was fixed in Lavdowsky's AFA fixative, and stained in Mayer's paracarmine as a whole mount preparation. When cross sectioned, it was stained in Ehrlich's acid hematoxylin and counterstained in eosin Y. All preparations were mounted in balsam.

Bialovarium Fischthal, 1953

DIAGNOSIS: Caryophyllaeidae with poorly defined scolex, bearing a pair of shallow loculi. Cirrus opens into utero-vaginal canal before it reaches the

*Contribution No. 6 from the Department of Biological Sciences, Harpur College, State University of New York, Endicott, New York.

surficial atrium. Ovary V-shaped and entirely medullary. Uterine coils extend only to lateral margins of cirrus sac, attaining a maximum longitudinal extent equivalent to one-fourth that of the testicular field. Terminal excretory bladder present. Seminal vesicle not inclosed in cirrus sac. Postovarian vitellaria absent. Parasitic in Cyprinidae. Development unknown.

Type Species: *Bialovarium nocomis* Fischthal, 1953

Bialovarium nocomis Fischthal, 1953

Diagnosis: With characters of genus. Body elongate; oval in cross section; anterior end bluntly rounded; width nearly uniform in testicular region, tapering only slightly to blunt point posteriorly; post-ovarian region extremely small (fig. 1). Scolex blunt, not distinct from neck, bearing a pair of shallow loculi. Cuticula thick. Subcuticular layer, between cuticula and outer longitudinal muscles, approximately of same thickness or thicker than cuticula. Inner and outer longitudinal muscles, originating in scolex and extending length of body, delineate medullary and cortical parenchyma, respectively; dorso-ventral muscle fibers present (figs. 2, 3, and 4). Terminal excretory bladder present.

Testes approximately 144 in number; usually transversely elongated; occur in one or two layers, somewhat in two longitudinal rows; entirely medullary (figs. 1 and 2). Vas deferens in middle portion of medullary parenchyma, between the rows of testes; anterior to and outside of cirrus sac the vas deferens enlarges into a relatively thick-walled seminal vesicle; short ejaculatory duct enters anterior end of cirrus sac. Cirrus sac large, nearly circular, surrounded by layers of circular muscles. Cirrus much convoluted, opening into utero-vaginal canal, within posterior end of cirrus sac; common genital atrium, very short, extending from this point to ventral body surface (figs. 1 and 3).

Ovary V-shaped, not lobed; wings extend antero-laterally from compact, oval ovarian mass medially located; entirely medullary; immediately behind cirrus sac (figs. 1, 4 and 5). Oöcapt located at antero-medial margin of oval ovarian mass. Oviduct short, thick-walled, surrounded on outside by gland cells; passes anteriorly a short distance before being joined by vagina. Junction point of oviduct and vagina forms vaginal-oviducal canal or fertilization chamber; canal passes anteriorly a short distance before being joined by common vitelline duct, then continues as uterus through highly glandular oötype. Uterus much convoluted lying entirely anterior to oval ovarian mass, between and anterior to ovarian wings, and lateral (but not anterior) to cirrus sac; joins vagina immediately behind posterior margin of cirrus sac, forming short utero-vaginal canal; uterus surrounded by many glands throughout entire length (figs. 1, 3, and 4). Vagina thick-walled, surrounded by unicellular glands along its length; becomes distended into thin-walled seminal receptacle a short distance before vagina joins oviduct .(figs. 1 and 4). Vitellaria entirely medullary; occur in one or two layers in two lateral rows, extending both anterior and posterior to testicular field; posterior limit at middle of sides of cirrus sac; follicles small compared to size of testes; post-ovarian vitellaria absent (figs. 1, 2 and 3).

Measurements in millimeters of the single whole mount specimen are: Body, length 4.55, width 0.60; anterior end of body to beginning of vitelline field, 0.28; anterior margin of vitelline field to anterior margin of testes, 0.30; anterior margin of cirrus sac to posterior end of body, 1.02; anterior margin of ovarian wings to posterior end of body, 0.87; longitudinal extent of vitelline field, 3.41; longitudinal extent of testicular field, 2.92; longitudinal extent

of uterine coils, 0.68; entire ovary, length 0.79; ovarian wings, length 0.63; median, oval ovarian mass, length 0.17, width 0.15. Measurements for 10 testes are: Length 0.069 (0.053-0.085), width 0.103 (0.078-0.120).

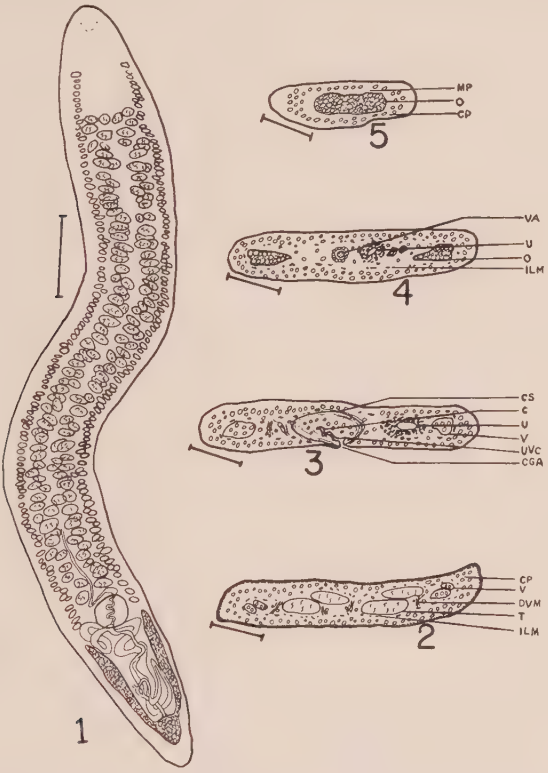
HOST: *Nocomis biguttatus* (Kirtland).

LOCALITY: Meadow creek, Barron County, Wisconsin, U. S. A.

HABITAT: Small intestine.

TYPE: Six slides of serial cross sections of entire worm in U. S. Nat. Mus. Helm. Coll. No. 48712.

Wardle and McLeod (1952) revised the classification of the family Caryophyllaeidae Leuckart, 1878, removing it from the order Pseudophyllidea, and raising it to the rank of an order, Caryophyllidea. The subfamilies Caryo-



Bialovarium nocomis

Drawn with the aid of a microprojector. The value of the scale is 0.5 mm. for Fig. 1 and 0.1 mm. for Figs. 2-5.

Fig. 1. Adult worm, ventral view.

Fig. 2. Cross section through testicular field.

Fig. 3. Cross section through region of cirrus sac at point of junction of cirrus and utero-vaginal canal.

Fig. 4. Cross section through region of ovarian wings.

Fig. 5. Cross section through region of medial, compact, oval ovarian mass.

C—cirrus
CGA—common genital atrium
CP—cortical parenchyma
CS—cirrus sac
DVM—dorso-ventral muscles
ILM—inner longitudinal muscles
MP—medullary parenchyma
O—ovary
T—testis
U—uterus and uterine glands
UVC—utero-vaginal canal
V—vitellaria
VA—vagina and vaginal glands

phyllaeinae Nybelin, 1922, Lytocestinae Hunter, 1927, Capingentinae Hunter, 1930, and Wenyoninae Hunter, 1927, are each elevated to the rank of a family, becoming Caryophyllaeidae, Lytocestidae, Capingentidae, and Wenyonidae, respectively. The respective subfamily definitions of Hunter (1930) are retained for these new families. The emendation of the subfamily Caryophyllaeinae by Fischthal (1951), probably appearing too late to be included by Wardle and McLeod in their book, would apply to their concept of the family Caryophyllaeidae. In the present paper Wardle and McLeod's revised classification is followed.

Hunter (1930) listed the following 7 genera in the subfamily Caryophyllaeinae: *Caryophyllaeus* Müller, 1787, *Monobothrium* Diesing, 1853, *Glaridacris* Cooper, 1920; *Caryophyllaeides* Nybelin, 1922, *Biacetabulum* Hunter, 1927, *Hypocaryophyllaeus* Hunter, 1927, and *Archigetes* Leuckart, 1878. Szidat (1938) added an eighth genus *Brachyurus*. Janiszewska (1950) presented a brief account of a ninth genus *Paraglaridacris;* although it was not placed in any subfamily nor the characteristics given by which the subfamily could be identified, it probably belongs to Caryophyllaeinae Nybelin, 1922, as the author indicated its position intermediate to *Glaridacris* and *Brachyurus*. Fischthal (1951) described a tenth genus *Pliovitellaria*. Wardle and McLeod (1952), in their newly created family Caryophyllaeidae, listed only the 7 genera of Hunter (1930). Although they discuss *Brachyurus* in connection with the genus *Archigetes,* they apparently do not give it the status of a separate genus.

The new genus *Bialovarium* differs from all known Caryophyllaeidae in having a V-shaped ovary; *Caryophyllaeides* has an ovary shaped like an inverted-A, while the other genera show a more or less H-shaped ovary. In possessing a scolex poorly defined from the neck *Bialovarium* differs further from *Glaridacris, Biacetabulum, Archigetes, Brachyurus* and *Paraglaridacris* which have well-defined scolices. In *Bialovarium* the cirrus opens into the uutero-vaginal canal before reaching the genital atrium, whereas it does not in *Caryophyllaeus, Monobothrium, Glaridacris,* and *Hypocaryophyllaeus;* this data is not given for *Paraglaridacris*. In *Bialovarium* the uterine coils extend only to the lateral margins of the cirrus sac, not anterior to it, as in *Caryophyllaeides, Biacetabulum, Hypocaryophyllaeus,* and *Archigetes*. *Bialovarium* possesses a seminal vesicle external to the cirrus sac, whereas *Caryophyllaeus* and *Caryophyllaeides* do not. Finally, *Archigetes* has a caudal vesicle carrying embryonic hooks, while *Bialovarium* has neither the vesicle nor the hooks.

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Transmission of Cattle Nematodes to Sheep

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In a recent paper, Porter (1953) reviewed the literature pertinent to the subject of cross transmission of helminths between cattle and sheep, and reported the results of original investigations. He found that *Haemonchus contortus* of bovine origin became established in appreciably greater numbers in calves than in lambs when both grazed together. *Cooperia punctata* and *C. pectinata* from cattle developed in numbers ranging from 9 to 30 times and 2 to 30 times greater, respectively, in calves than in lambs. Three other species of cattle parasites, *Oesophagostomum radiatum, Nematodirus helvetianus* and *Ostertagia ostertagi,* were not found in lambs killed 19 to 35 days after their exposure on a pasture contaminated by cattle harboring these helminths.

On the basis of these findings, there would appear to be a beneficial effect from grazing sheep and cattle together, as suggested by Snell (1936). It was not known, however, whether sheep are also refractory to the immature stages of those cattle nematodes which Porter (1953) did not find as adults in sheep. Since much of the experimental work on the pathology and pathogenicity of nematode parasitism of cattle and sheep has shown that the greatest injury is inflicted by the immature worms (Andrews, 1942; Mayhew, 1944; Herlich and Porter, 1953; Todd *et al.,* 1951, 1953), it was considered important to determine whether those cattle nematodes not normally found as adults in sheep, could maintain themselves in the ovine host for a short time and perhaps produce a pathology similar to that observed in cattle.

PROCEDURE

The tests were conducted from June to September 1953 on a 39- by 63-foot Bermuda grass lot, which had been experimentally contaminated with manure collected from naturally-parasitized calves. Examinations of this manure showed low-grade infections with most of the nematode species common to the southeastern United States.

The animals used were grade Jersey calves and grade Southdown lambs that had been raised parasite-free, except for *Eimeria* spp. and *Strongyloides papillosus.* The animals were from 13 to 20 weeks old. A calf and lamb of similar age were paired for each trial. Three calf-lamb pairs were placed on the lot, one pair at a time, 3, 24 and 66 days, respectively, after the last application of manure to the lot. The oldest pair was placed on the lot first, and the youngest pair last. After grazing for 15 days, each calf-lamb pair was killed for post-mortem examination.

Fecal examinations were made by the D.C.F. technic, and the numbers of worms recovered were estimated by means of dilution counts. The gastrointestinal tracts were examined for gross pathology, and the numbers of nodules associated with *Oes. radiatum* were actually counted.

RESULTS AND DISCUSSION

The kinds and numbers of nematodes recovered from the experimental animals are recorded in Table 1. All specimens were immature, except in the cases of *C. punctata* and *C. pectinata,* which were present both as adults and larvae. Since no specimens of *Oes. radiatum* were recovered from the in-

testinal lumen of any animal, the nodules were counted and are recorded in Table 1.

All lambs were free of *C. pectinata,* which was present in all 3 calves, indicating that sheep are highly refractory to this parasite. *C. punctata,* on the other hand, was recovered in greater numbers from Lambs 1 and 2 than from the calves with which they were paired. In view of Porter's (1953) finding of 9 to 30 times more adult *C. punctata* in calves than in lambs, it is of particular interest that in these trials 52% of the specimens found in the calves were fifth-stage worms, adolescents and adults, whereas only 36% in the lambs were beyond the fourth stage. In addition, only Lamb 2 passed Cooperia eggs in 15 days, whereas all calves passed eggs by the fourteenth day. These observations suggest either that *C. punctata* has a longer prepatent period in sheep than in calves, or that as the worm nears maturity in sheep, it is eliminated from the body.

TABLE 1.—Data on calves and lambs exposed to nematodes from cattle.

Animal No.	Age (Weeks)	Time on Lot	Number of nematodes recovered					Weight Changes		
			Haemonchus contortus	*Ostertagia ostertagi*	*Cooperia punctata*	*Cooperia pectinata*	*Oes. radiatum* (nodules)	On	Off	Diff.
Calf 1303	18½		325	150	14,000	800	342	191	190	— 1
Lamb 1	20	6/29-7/14	275	50	16,400	0	64	60	55	— 5
Calf 1305	18		870	60	80,100	1,800	686	175	165	—10
Lamb 2	19	7/20-8/4	360	0	130,800	0	527	29	27	— 2
Calf 1307	15		120	60	8,100	900	97	150	147	— 3
Lamb 3	13	8/31-9/15	0	0	1,320	0	0	35	28	— 7

H. contortus, Oes. radiatum and *O. ostertagi* were present in the 3 calves and in Lamb 1, but only the first 2 species were found in Lamb 2 and all were absent from Lamb 3. In all cases, infections with the stomach worms, *H. contortus* and *O. ostertagi,* were of a low order, and no evidence of gross pathology was found. The nodules found in the small and large intestines as a result of tissue penetration by the larvae of *Oes. radiatum* were comparable in size in all 3 calves. In Lamb 1 the nodules were of a size equal to those seen in the calves, but in Lamb 2 they were noticeably smaller; they were entirely absent in Lamb 3. The fact that Lamb 3 had none of the 3 aforementioned species of worms may have been a result of a decrease in available infective larvae coincident with a decrease in forage available for grazing. However, the following hypothesis is predicated as an explanation for the total absence of these 3 species: Fresh larvae possess a greater degree of virility and infectivity enabling them to maintain themselves, at least for short periods, in an abnormal host, while older larvae exposed to the detrimental effects of climate, especially desiccation, lose their ability to infect any host other than the normal one. The temperature ranged from a minimum of 52° F. to a maximum of 99° F. during June to September, well within the normal temperature for that time. Precipitation was normal for the test period, except in August when only 3.76 inches of rainfall were recorded, this being 0.83 inches below normal.*

None of the animals exhibited pronounced symptoms of parasitism; however, as is shown in Table 1, all lost a few pounds in weight. Such slight loss is not unusual during the first 2 weeks after being placed on pasture.

*Climatological Data, Alabama, U.S. Weather Bureau, *59*(6,7,8,9) 1953.

The results of these tests indicate that grazing sheep and cattle together might result in detrimental effects on sheep, since the larval stages of all the cattle parasites discussed in this paper are capable of maintaining themselves for short periods in lambs, although unable to reach sexual maturity in some cases. Since eggs of these parasites would not be passing in the feces, this type of parasitism would be particularly insidious inasmuch as diagnosis, difficult under ordinary circumstances, would be impossible by fecal examination. Whether sheep parasites, not normally found in cattle, are also able to maintain themselves for short periods in cattle, remains to be determined.

SUMMARY

A small experiment is described in which it was determined that the larval stages of *O. ostertagi* and *Oes. radiatum,* parasites of cattle not normally found as adults in sheep, were able to maintain themselves for short periods in sheep. Tissue penetration and gross pathology similar to that observed in cattle also were observed in sheep that acquired *Oes. radiatum.* An hypothesis is advanced to account for the complete absence of bovine parasites in one of the 3 ovines tested, namely, that fresh infective larvae are better able to maintain themselves in an abnormal host than older larvae exposed to adverse climatic effects, and that the older larvae lose their ability to infect animals other than their usual host.

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A Pseudophyllidean Tapeworm from a Dog in Southeastern United States*

R. E. THORSON and E. M. JORDAN

A female collie was presented at the Alabama Polytechnic Institute small animal clinic for an ovariohysterectomy. On routine fecal examination pseudophyllidean eggs were observed. The eggs resembled those of *Diphyllobothrium latum* but had more pointed ends. The measurements of one egg were 61 by 37 microns. One pseudophyllidean tapeworm recovered following treatment was provisionally identified as *Spirometra mansonoides.* This provisional diagnosis was supported by Allen McIntosh of the Agricultural Research Service, USDA. Considerable difficulty was experienced in identi-

*Approved by the Committee on Publications, School of Veterinary Medicine, Alabama Polytechnic Institute. Publication No. 430.

fication due to the fact that there were no gravid segments in the worm which was recovered. The dog was reared in Alabama and taken to Columbus, Georgia. According to the owner the dog had never been seen eating fish or snakes but was seen eating a rat. This appears to be the first record of this form in either Alabama or Georgia.

Report of the Brayton H. Ransom Memorial Trust Fund

FUNDS ON HAND, Jan. 1, 1953	$1,751.36
RECEIPTS: Interest rec'd in 1953	59.59
DISBURSEMENTS: Expenses and grant to Helminthological Society of Washington	56.00
BALANCE ON HAND, Dec. 31, 1953	$1,754.95

ELOISE B. CRAM,
Secretary-Treasurer

Minutes

Three Hundred Seventeenth to Three Hundred Twenty-fourth Meetings

317th meeting: McMahon Hall, Catholic University of America, October 21, 1953. Dr. Sarles was appointed as member of the Executive Committee to replace Dr. Dikmans who has resigned. Ten dollars was voted for the Science Fair of the Washington Jr. Academy of Science. PAPERS PRESENTED: Report on the International Zoological Congress, Copenhagen 1953, by Steiner, The Genus *Peripatus* by Lynn, a Review of Boedenheimer's book "Insects as Human Food" by Reinhard.

318th meeting: Library of the Zoological Division of the B.A.I., Beltsville, Md., November 20, 1953. PAPERS PRESENTED: Effect of Enheptin on the host-parasite relationship in blackhead of turkeys by Tromba, Correlation of growth rate and severity of cecal lesions in chicks by Gardiner, Liverfluke infections in sheep by Becklund, Bacteria-free cultures of trichomonads by Diamond, Cultivation of *Entodinium* from California deer by Sayama, Kidney worm control with a Borax compound by Alicata, Current status of our knowledge of *Leucocytozoon* in waterfowl (Anatidae) by Herman, Parasitism in cattle in the Southeast by Andrews.

319th meeting: McMahon Hall, Catholic University of America, December 16, 1953. Dr. Reinhard and Mr. Taylor were appointed to the Editorial Board. Officers elected for the year 1954 were Dr. M. Sarles, President; Dr. F. Enzie, Vice President; Miss Edna Buhrer, Corresponding Secretary and Treasurer; Dr. G. Luttermoser, Recording Secretary. PAPERS PRESENTED: Tissue culture studies on the exo-erythrocytic forms of *Plasmodium gallinaceum* by Dubin, Typical life-history of a coccidium of sheep by Lotze, and Electrophoretic studies of the blood of rats infected with *Nippostrongylus muris* by Dr. S. E. Leland, Jr., University of Kentucky.

320th meeting: Biology Building, University of Maryland, January 20, 1954. Drs. Rozeboom and Stirewalt were appointed as members of the Executive Committee. PAPERS PRESENTED: Japanese film on Tsutsugamushi fever directed by Dr. M. Sossa, University of Tokyo, Morphology of chigger mites by Brown, Biting habits of the chiggers *Trombicula splendens* and *T. alfreddogesi* by Cross, The mechanism by which chiggers feed on mammals and man by Wharton.

321st meeting: Medical Service Graduate School, Walter Reed Army Medical Center, February 17, 1954. PAPERS PRESENTED: Studies on control of chigger vectors of Scrub Typhus in Borneo by Traub, Notes on the land

leeches of Southeast Asia by Walton, A new antigen for Complement Fixation tests in schistosomiasis by Kent, A report on Schistosomiasis Symposium, 8th Pacific Science Congress by McMullen.

322nd meeting: National Institutes of Health, Bethesda, Md., March 19, 1954. PAPERS PRESENTED: Suppression and cure of malaria by Coatney, Recent studies of metabolism of trypanosomes by von Brand, New evidence of the importance of *Toxoplasma* in ocular disease by Jacobs, The effect of Cortisone on immune response of the white rat to *Nippostrongylus muris* by Weinstein.

323rd meeting: Georgetown University Medical School, April 21, 1954. Editorial Committee reported that Dr. G. F. Otto would continue as Editor of the Proceedings of the Society. PAPERS PRESENTED: Histochemical studies of schistosomes by Lt. P. K. Hamilton and Dr. F. Johnson, Effect of snail maintenance temperatures on development of *Schistosoma mansoni* by Stirewalt, Some effects of spiral nematodes on ornamental plants by Golden and Korean Hemorrhagic Fever by Lt. Col. L. Zimmerman.

324th meeting: School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md., May 21, 1954. The W. W. Cort Library of the Parasitology Department was dedicated in honor of Doctor W. W. Cort, Emeritus Professor of Parasitology and Life Member of this Society. PAPERS PRESENTED: Sex difference in susceptibility of hamsters to *Nippostrongylus muris* by A. J. Haley, Miracidia immobilization reaction in *Schistosoma mansoni* infections in the hamster by L. Senterfit, Inheritance of yellow orange fat color in *Culex pipiens* by Spielman, Hybridization of *Aedes pseudoscutellaris* and *Aedes polynesiensis* by Rozeboom, Protein Complexes in Cestodes by F. M. Kent.

THE FOLLOWING WERE ELECTED TO MEMBERSHIP DURING THE YEAR: 317th meeting: Willard W. Becklund, Zacarias De Jesus, T. Bonner Stewart, Frank A. Ehrenford, Halsey H. Vegoos, Harold W. Reynolds; 318th, F. B. Bang, A. James Haley, Robert G. Leek, Robert Rubin, I. Barry Tarshis, G. W. Wharton, Grant I. Wilson; 319th, Ralph F. Honess, Kenji Sayama; 320th, Thomas K. Sawyer; 321st, George R. LaRue, W. J. Van derLinde, Philip H. Santmyer, Frans C. Goble, P. N. Drolsom; 322nd, Satyu Yamaguti, Westen J. Martin, Calvin L. Massey, Isaias Tagle V., Thomas A. O'Keefe, Ralph E. Duxbury, J. E. Bosher; 323rd, Muneo Yokogawa, Joseph M. Good, Howard R. Garriss, H. F. Hsü; 324th, Lt. John C. Hall, Clyde M. Singer, Albert W. Grundman.

GEORGE W. LUTTERMOSER,
Recording Secretary

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